

BIRLA CENTRAL LIBRARY
PILANI [RAJASTHAN]

R

Class No. 616.01

Book No. S989 V. 8

Accession No. 59201



Privy Council

MEDICAL RESEARCH COUNCIL

**A SYSTEM OF
BACTERIOLOGY
IN RELATION TO
MEDICINE**

VOLUME VIII

**LONDON :
PUBLISHED BY HIS MAJESTY'S STATIONERY OFFICE**

1931

MEDICAL RESEARCH COUNCIL

The Right Hon. the Viscount D'ABERNON, G.C.B., G.C.M.G., LL.D.

(*Chairman*).

The Right Hon. LORD MILDMAY OF FLETE, P.C. (*Treasurer*).

A. G. CHURCH, D.S.O., M.C., B.Sc., M.P.

Professor T. R. ELLIOTT, C.B.E., D.S.O., M.D., F.R.S.

Professor J. B. LEATHES, B.M., F.R.C.S., F.R.S.

Professor ROBERT MUIR, M.D., Sc.D., F.R.S.

SIR JOHN H. PARSONS, C.B.E., D.Sc., F.R.C.S., F.R.S.

Professor J. J. R. MACLEOD, M.B., D.Sc., F.R.S.

WILFRED TROTTER, M.S., F.R.C.S.

SIR CHARLES SHERRINGTON, O.M., G.B.E., M.D., Sc.D., F.R.S.

J. A. ARKWRIGHT, M.D., F.R.S.

SIR WALTER M. FLETCHER, K.B.E., C.B., M.D., Sc.D., F.R.S.

(*Secretary*).

BACTERIOLOGICAL COMMITTEE

Professor WILLIAM BULLOCH, M.D., LL.D., F.R.S. (*Chairman*).

SIR FREDERICK W. ANDREWES, D.M., F.R.S.

Captain S. R. DOUGLAS, M.R.C.S., F.R.S., late I.M.S. (*Joint Secretary*).

Professor GEORGES DREYER, C.B.E., M.D., F.R.S.

PAUL FILDES, O.B.E., M.B. (*Joint Secretary*).

W. E. GYE, M.D.

P. P. LAIDLAW, B.Ch., F.R.S.

Professor J. C. G. LEDINGHAM, C.M.G., D.Sc., M.B., F.R.S.

Professor W. W. C. TOPLEY, M.D.

Dr. PAUL FILDES and Professor J. C. G. LEDINGHAM, F.R.S., have acted on behalf of the Committee as general editors of this System of Bacteriology. The chief burdens of its preparation and of other tasks of organization on behalf of the Council have fallen upon Dr. FILDES and Dr. EDGAR SCHUSTER.

For this Volume Dr. P. FILDES, Dr. E. HINDLE, Professor R. T. HEWLETT and Professor J. C. G. LEDINGHAM have assisted in the editorship with regard to particular sections.

A
SYSTEM OF BACTERIOLOGY
IN RELATION TO MEDICINE

VOLUME VIII

BY

W. BULLOCH, L. COLLBROOK, J. CRUICKSHANK, R. CRUICKSHANK,
A. DAWSON, J. T. EDWARDS, F. GRIFFITH, L. W. HARRISON,
E. HINDLE, IVY MACKENZIE, A. ROBERTSON,
R. ST. JOHN-BROOKS

CONTENTS

	PAGE
CHAPTER I. FUNGI PATHOGENIC TO MAN.. .. .	11
GENERAL ACCOUNT	11
Introduction	11
General characters of fungi	12
Classification	12
<i>Phycomycetes</i> , 12; <i>Ascomycetes</i> , 13; <i>Basidiomycetes</i> , 13; <i>Fungi imperfecti</i> , 13.	
Biological phenomena	14
<i>Homothallism and heterothallism</i> , 14; <i>Polymorphism and pleomorphism</i> , 14; <i>Variation</i> , 15; <i>Strains</i> , 15; <i>Saltation (mutation)</i> , 15.	
Technique	16
<i>Culture media</i> , 16; <i>Examination</i> , 16.	
Occurrence	16
Poisonous fungi	17
Nomenclature	17
PHYCOMYCETES	18
Chytridiales	18
<i>Rhizosporidium</i> , 18; <i>Coccidioides</i> Rixford and Gilchrist, 19.	
Zygomycetes: Mucorineæ	20
<i>Mucor Micheli</i> , 21; <i>Rhizopus</i> Ehrenberg, 22; <i>Absidia</i> van Tieghem, 23.	
ASCOMYCETES	23
Saccharomycetaceæ and yeast-like fungi	24
<i>Saccharomyces</i> Meyen, 26; <i>Debaromyces</i> Klöcker, 27; <i>Wilha Hansen</i> , 27; <i>Cryptococcus</i> Kützing emend. Vuillemin, 27; <i>Endomyces</i> Rees, 28; <i>Momilia</i> Auct., 29; <i>Enanthiothamnus</i> Pinoy, 31.	
Plectascineæ	31
<i>Allescheria</i> Saccardo & Sydow (<i>Eurotiopsis</i> Cost.), 32.	
Asterineæ	33
FUNGI IMPERFECTI	34
Hyphomycetes: Thallosporeæ	36
Hyphomycetes: Blastosporeæ	36
<i>Phialophora</i> Medlar, 36; <i>Blastocystis hominis</i> Brumpt, 37.	
Hyphomycetes: Arthrosporeæ	37
<i>Oidium</i> Auct., 37; <i>Trichosporum</i> Behrend, 38; <i>Torula</i> Persoon emend. Sacc., 39; <i>Madurella</i> Brumpt emend. Pinoy, 39; <i>Indiella</i> Brumpt, 40; <i>Glenospora</i> Vuillemin, 40; <i>Scedosporium</i> Saccardo, 41.	
Hyphomycetes: The Ringworm Group	41
Hyphomycetes: Hemisporæ	41
<i>Hemispora</i> Vuillemin, 41.	
Hyphomycetes: Conidiosporeæ	42
(Sporotrichæ) <i>Sporotrichum</i> Auct., 43; <i>Trichosporium</i> Fries, 43.	
(Sporophoræ) <i>Acremonium</i> Link, 44.	
(Phialideæ) <i>Aspergillus</i> , 44; <i>Penicillium</i> , 45; <i>Scopulariopsis</i> Bainier emend. Thom, 46.	
THE RINGWORM GROUP	46
Pathology	46
<i>Introduction</i> , 46; <i>History</i> , 47; <i>The Microsporum group</i> , 47; <i>The Endothrix group</i> , 49; <i>The Ectothrix group</i> , 52; <i>The Epidermophyton group</i> , 55; <i>The Endodermophyton group</i> , 56; <i>The Favus group</i> , 56.	
Immunity, sensitization and systematic infections	57
Cultivation	58
Classification	59
Pleomorphism	62
Physiology	67

	PAGE
CHAPTER II. ACTINOMYCOSIS AND SIMILAR CONDITIONS ..	71
HISTORY OF ACTINOMYCOSIS	71
THE AEROBIC ACTINOMYCES	72
Nomenclature	72
Ecology	72
Principal characters	73
Infections due to or associated with aerobic <i>Actinomyces</i>	73
<i>Mycetoma</i> , 73; <i>Cattle farcy</i> , 77; <i>Other actinomycotic infections of man and animals</i> , 77.	
Systematic position of genus <i>Actinomyces</i>	78
THE ANAEROBIC ACTINOMYCES	78
<i>The characters of Actinomyces bovis</i> , 79; <i>Pathogenicity of Actinomyces bovis</i> , 80; <i>The ætiology of actinomycosis</i> , 82; <i>The detection and isolation of Actinomyces bovis</i> , 84; <i>Further work required in connection with actinomycosis and allied infections</i> , 85.	
<i>Bacterium actinomycetem comitans</i>	85
ACTINOBACILLOSIS	87
<i>Distribution of actinobacillosis</i> , 87; <i>Description of lesions</i> , 87; <i>The characters of the Actinobacillus</i> , 88; <i>Pathogenicity</i> , 88.	
CHAPTER III. PATHOGENIC LEPTOTHRICEÆ	91
<i>General description and classification</i> , 91; <i>Pathogenicity</i> , 93; <i>Leptothrix meningitis</i> , 94; <i>The writer's observations</i> , 96; <i>Conclusions</i> , 98.	
CHAPTER IV. THE SPIROCHÆTES	101
INTRODUCTION	101
GENERAL DESCRIPTION	102
<i>Definition</i> , 102; <i>Form</i> , 102; <i>Movement</i> , 103; <i>Size</i> , 104.	
NOMENCLATURE AND CLASSIFICATION	104
<i>Classification here adopted</i> , 105; <i>Noguchi's classification</i> , 106.	
REPRODUCTION	107
GENERAL CONDITIONS OF LIFE	110
CULTIVATION	112
FILTERABILITY	115
PATHOGENIC ACTION	115
IMMUNITY	119
<i>Natural immunity</i> , 119; <i>Acquired immunity</i> , 120; <i>The properties of immune serum</i> , 121; <i>Passive immunity. Serum therapy</i> , 124; <i>Vaccination</i> , 124; <i>Toxins</i> , 124.	
CHEMOTHERAPY	124
FREE-LIVING SPIROCHÆTES	126
COMMENSAL SPIROCHÆTES FROM PLANTS AND ANIMALS	128
GENERA ALLIED TO SPIROCHÆTES	130
<i>Heliconema Sanarelli</i> , 1927, 130; <i>Cristispiraella Hollande</i> , 1921, 131; <i>Spiraella Duboscq and Lebailly</i> , 1912 ¹ , 131; <i>Paraspirillum Dobell</i> , 1911, 132; ' <i>Spirochæta</i> ' <i>cytophaga Hutchinson and Clayton</i> , 1919, 132.	
SPIROCHÆTES OF DOUBTFUL SIGNIFICANCE IN HUMAN DISEASE	133
<i>Spirochæta argentinensis Kuhn and Steiner</i> , 1917, 133; <i>The spirochæte of mumps</i> , 134.	
CHAPTER V. COMMENSAL SPIROCHÆTES	141
<i>Spirochætes of the human mouth</i> , 141; <i>Intestinal spirochætes</i> , 143; <i>Ulcus tropicum and spirochætes found in the skin</i> , 144; <i>Spirochætes found in the genitalia</i> , 145.	

	PAGE
✓CHAPTER VI. BLOOD SPIROCHÆTES	147
INTRODUCTION	147
GENERAL ACCOUNT	147
Morphology	148
Staining	149
<i>Fontana's method</i> , 150; <i>Dobell's modification of Levaditi's silver impregnation method</i> (Wenyon, 1926), 150.	
Cultivation	151
✓ Pathogenic action	152
Immunity	155
Brain infections	156
Therapeutic use of relapsing-fever infections	157
✓ Treatment	158
Method of transmission	158
<i>Transmission of spirochætes by O. moubata</i> , 159; <i>Transmission of spirochætes by Pediculus humanus</i> , 161.	
Animal reservoirs	163
VARIETIES OF HUMAN RELAPSING FEVER	164
Europe	164
<i>European relapsing fever (S. recurrentis Lebert, 1874)</i> , 164; <i>Spanish relapsing fever (S. hispanica de Buen, 1926)</i> , 165.	
Asia	165
<i>Indian relapsing fever (S. carteri Mackie, 1907)</i> , 165; <i>Quetta relapsing fever</i> , 166; <i>Persian relapsing fever (S. persica Dschunkowsky, 1913)</i> , 166; <i>Bokharan relapsing fever (S. usbekistanica Pickoul, 1928)</i> , 167; <i>Relapsing fever in Asia Minor</i> , 167; <i>Relapsing fever in Eastern Asia</i> , 167.	
Africa	168
<i>North African relapsing fevers</i> , 168; <i>The relapsing fevers of Tropical Africa</i> , 169.	
America	170
<i>North American relapsing fever (S. novyi Schellack, 1907)</i> , 170; <i>South American relapsing fevers (S. venezuelensis Brumpt, 1921 and S. neotropicalis St. John and Bates, 1922)</i> , 171.	
Australasia	171
BLOOD SPIROCHÆTES OCCURRING IN ANIMALS	171
Blood spirochætes occurring in mammals	172
<i>Primates</i> , 172; <i>Chiroptera (Bats)</i> , 172; <i>Carnivora</i> , 172, <i>Insectivora</i> , 173; <i>Rodentia</i> , 173; <i>Ungulata</i> , 174; <i>Marsupialia</i> , 176.	
Blood spirochætes occurring in birds	176
<i>Spirochæta anserina Sakharoff, 1891</i> , 176; <i>Other spirochætes recorded from birds</i> , 179.	
Blood spirochætes occurring in cold-blooded animals	180
CHAPTER VII. SYPHILIS	185
HISTORY	185
NOMENCLATURE	186
GENERAL CHARACTERS OF <i>S. PALLIDA</i> AND ITS RELATION TO OTHER ORGANISMS	187
✓MORPHOLOGY	188
Motility	188
Staining	188
Involution forms	189
CULTIVATION	191
Primary culture	192
Isolation in pure culture	193
Cultural characteristics	194

	PAGE
DISTRIBUTION AND PERSISTENCE	195
Viability	195
Distribution in tissues of animals	196
Distribution in man and persistence of infectivity	197
✓ PATHOGENIC ACTION	197
Methods of transference	197
Speed of invasion and modes of spread in the tissues	199
Susceptibility of experimental animals	199
Pathogenic action on rabbits	200
<i>Intratesticular inoculation, 200 ; Intradermal inoculation, 202 ; Lesions subsequent to the primary, 202 ; Metastatic orchitis, 202 ; Invasion of blood-stream, glands and other tissues, 202.</i>	
Pathological results in man	204
<i>Histological changes at sites of inoculation, 204 ; Changes in the regional lymphatics and glands, 204 ; Spread to other tissues, 205 ; The skin and mucous membranes, 205 ; Subcutaneous tissues, muscles, bones and joints, 206 ; Viscera, 207 ; Genital organs, 208 ; Bladder, 208 ; Stomach and intestines, 209 ; Lungs, 209 ; Cardiovascular system, 209 ; Blood changes, 211 ; The central nervous system, 212 ; Recognition of syphilis in a macerated fœtus, 216.</i>	
RESISTANCE	219
Natural resistance	219
<i>Non-reaction at site of inoculation, 219 ; Circumstances affecting the development of a primary lesion, 219.</i>	
Acquired resistance	220
<i>Conditions affecting the acquisition of resistance, 221 ; Resistance to superinfection and to reinfection in man, 222 ; Superinfection and reinfection in animals, 224.</i>	
Summary	228
ANTIBODIES IN THE SERUM	229
Agglutinins	229
Spirochæticidins	230
Effects of ' antisera ' in prophylaxis and treatment	230
Spirochætolysins	230
Complement fixation and precipitation	231
Summary	237
PRACTICAL DIAGNOSIS	239
Microscopical examination	239
Serum tests	241
<i>General principles of complement-fixation tests, 241 ; Complement-fixation tests on the principles of Hecht and of Stern, 241 ; Tests on the principle of the original Wassermann reaction, 241 ; Tests depending on directly visible effects of interaction between serum and extract, 251 ; Diagnostic value of the Wassermann and flocculation tests of the blood-serum, 257.</i>	
Tests of the cerebrospinal fluid	262
<i>Complement fixation, 262 ; Flocculation reactions, 263 ; Cytological examination, 264 ; Supplementary reactions, 265 ; Effect of treatment on the different pathological reactions of the cerebrospinal fluid, 266.</i>	
The relation between serum and spinal fluid reactions	267
Gutaneous tests	268
Post-mortem examination	268
TREATMENT	271
Chemotherapy	271
<i>Mercury, 271 ; Arsenicals, 271 ; Bismuth, 274 ; Compounds of bismuth and arsenic, 275 ; The salts of other metals, 275.</i>	
Pyrogenetic therapy	275
<i>Treatment with malarial fever, 276 ; The use of other pyrogenetic agents, 277 ; Mode of action, 277.</i>	

CONTENTS

9

	PAGE
SPIROCHÆTA CUNICULI NOGUCHI (1921)	283
<i>Pathogenicity</i> , 283 ; <i>Cultivation</i> , 284 ; <i>Treatment</i> , 285.	
CHAPTER VIII. RAT-BITE FEVER OR SODOKU	286
<i>Clinical</i> , 286 ; <i>Ætiology</i> , 287 ; <i>The causative organism</i> , 288 ; <i>Geographical distribution and vectors</i> , 290 ; <i>Pathology</i> , 291 ; <i>The disease in animals</i> , 292 ; <i>Serology</i> , 294 ; <i>Treatment</i> , 295.	
CHAPTER IX. THE LEPTOSPIROSES	296
GENERAL ACCOUNT	296
SPIROCHÆTA (LEPTOSPIRA) BIFLEXA WOLBACH & BINGER, 1914 ..	297
The pathogenicity of water leptospira	298
INFECTIOUS JAUNDICE AND OTHER LEPTOSPIRAL INFECTIONS	301
SPIROCHÆTA (LEPTOSPIRA) ICTERO-HÆMORRHAGIÆ INADA AND IDO, 1915	301
Cultivation	301
<i>Media</i> , 301 , <i>Isolation of Leptospira in cultures</i> , 302.	
Pathological effects	303
<i>In man</i> , 303 ; <i>In animals</i> , 304 ; <i>General pathological changes produced</i> , 307.	
Immunological reactions	308
Immunity	310
Serum therapy.. .. .	310
Chemotherapy	311
SPIROCHÆTA (LEPTOSPIRA) AUTUMNALIS	312
SPIROCHÆTA (LEPTOSPIRA) HERDOMADIS IDO, ITO AND WANI, 1918 ..	313
SPIROCHÆTA (LEPTOSPIRA) PYROGENES VERVOORT, 1923	314
DOUBTFUL FORMS	314
<i>Spirochæta (Leptospira) hæmoglobinuriæ</i> Schuffner, 1918	314
<i>Spirochæta bilohæmoglobinuriæ</i> Blanchard and Lefrou, 1922	314
<i>Spirochæta (Leptospira) sp</i>	315
<i>Spirochæta (Leptospira) pettiti</i> Fiessinger, 1918	316
<i>Spirochæta (Leptospira) interrogans</i> Stimson, 1909	316
<i>Spirochæta (Leptospira) cowyi</i> J. Gomes de Faria, 1924	317
CHAPTER X. YAWS	320
HISTORY	320
GENERAL DESCRIPTION OF THE CAUSATIVE ORGANISM AND ITS RELATION TO OTHER ORGANISMS	320
STAINING AND CULTIVATION	320
DISTRIBUTION AND PERSISTENCE	321
Viability	321
Distribution in animals	321
Distribution in man	321
PATHOGENIC ACTION	322
Susceptibility of experimental animals	322
Pathology in man	324
<i>Method of transference</i> , 324 ; <i>Modes of spread and distribution in the body</i> , 324 ; <i>Pathological results in man</i> , 325 ; <i>Evidence of responsibility of the organism for the disease</i> , 325 ; <i>Evidence of responsibility for accessory diseases</i> , 326.	
RESISTANCE	326
PRACTICAL DIAGNOSIS	327
Detection of the organism	327
Indirect diagnosis	327
<i>The Wassermann reaction</i> , 327 ; <i>Flocculation tests</i> , 328 ; <i>Cutaneous test</i> , 328.	

	PAGE
IMMUNIZATION	328
THE IMMUNOLOGICAL RELATIONSHIP OF YAWS AND SYPHILIS	330
CHEMOTHERAPY	332
ANTIBACTERIAL MEASURES	332
 CHAPTER XI. THE NORMAL BACTERIAL FLORA OF MAN ..	 334
THE BACTERIAL FLORA OF THE CUTANEOUS SURFACES	334
BACTERIAL FLORA OF THE NORMAL MOUTH	336
<i>B. fusiformis</i>	339
THE BACTERIAL FLORA OF THE UPPER RESPIRATORY TRACT	343
The vestibule and nasal fossæ	343
<i>The elimination of bacteria from the nose</i>	345
Nasopharynx	346
The trachea and bronchi	347
The Gram-negative cocci of the nasopharynx	347
THE BACTERIAL FLORA OF THE NORMAL GASTRO-INTESTINAL TRACT ..	350
Introduction	350
Development of the intestinal flora	351
Division of intestinal flora into different groups	352
<i>The coliform group, 353; The aciduric group of Gram-positive bacilli, 354; The Gram-positive cocci, 354; Spore-bearing bacteria, aerobic and anaerobic, 355; Spirochaetes, 355; Moulds and yeasts, 356; An alternative method of classification, 356.</i>	
The bacterial flora at different levels of the intestine of the adult ..	356
Factors which influence the characters of the intestinal flora	358
The function of the intestinal bacteria	360
THE BACTERIAL FLORA OF THE VAGINA	363
THE ACIDURIC GROUP OF INTESTINAL ORGANISMS	369
<i>B. bifidus communis</i> Tissier	370
<i>B. acidophilus</i> Moro	372
Döderlein's bacillus	375
 CHAPTER XII. SWINE ERYSIPELAS	 379
<i>Introduction, 379; The disease in the pig, 380; The bacillus of swine erysipelas, 380; Immunity, 385; Similar infections in species other than the pig, 387.</i>	

CHAPTER I. FUNGI PATHOGENIC TO MAN.

By J. RAMSBOTTOM (BRITISH MUSEUM, NATURAL HISTORY) ;
WITH A SECTION BY ARTHUR WHITFIELD (LONDON).

General Account.

INTRODUCTION.

THE space allotted to mycology is so restricted that it is impossible here to cover the field even in summary. The treatment adopted consequently needs a little explanation. It seemed most profitable to enter into certain matters such as classification and cultural characters in some detail so as to emphasize the difference in outlook of the mycologist and the bacteriologist. In some of the larger groups, rather than attempt a summary of present knowledge I have sought to suggest how further precise knowledge can best be acquired. Occasionally when it has appeared advantageous a detailed account of a fungus is given to serve as a pattern of the type of diagnosis which should prove satisfactory.*

The arrangement adopted is systematic, the different fungal groups being considered in order. In many ways it would have been advantageous to deal directly with the diseases, but this would entail much repetition. Thus, for example, 'Maduromycosis' has been recorded as due to species of *Madurella*, *Indiella*, *Glenosporium*, *Scedosporium*, *Allescheria*, *Aspergillus*, *Penicillium*, *Sporotrichum*, *Torula* and *Mucor*, many of which genera are taxonomically unrelated.

Only those fungi reputed to be pathogenic to man are considered ; to extend the treatment to animals generally would necessitate the inclusion of a much larger number of genera and many general points in biology.

Fungi are responsible for a number of well-known diseases. Recent investigations suggest that fungal diseases are far more prevalent than was formerly supposed, and that certain obscure maladies will prove to be due to them. There is, however, a tendency on the part of some investigators to assume a fungal origin of a disease on insufficient evidence. It is not enough to postulate the parasitic nature of a disease and then, bacteria, protozoa and other parasites being eliminated, to describe it as mycotic ; the evidence must be as clear as that for any other parasitic group. The need for saying this is doubtless due to the general lack of training in mycology amongst those studying human and animal diseases. Correlated with this lack of appreciation for the need of something more definite than a residuum diagnosis is the uselessness of many of the

* The measurements given in some of the descriptions are of an unnecessary refinement.

figures which are supposed to illustrate the morphology of pathogenic fungi. It is impossible even to guess what these figures represent, and copied into text-books they add to the confusion.

Another matter which calls for comment is the casual way in which new names are often coined. Here also is a suggestion of a vicious circle. There is an intrinsic difficulty presented by the classification of pathogenic fungi, but this is added to by poor description and waywardness in naming. Many generic and specific diagnoses conform in no way to what a mycologist would consider necessary ; in fact, they resemble those which were sometimes given when mycology was in its infancy. The same carelessness is shown in the proposal of new generic names for an anomalous species with a total disregard of any previous transference (or transferences), and a consequent litter of synonyms. It is not to be assumed, however, that all difficulties in nomenclature arise thus, for a good proportion are due to the historical development of the subject.

GENERAL CHARACTERS OF FUNGI.

Fungi resemble bacteria in many ways, the most important of which is the lack of chlorophyll, the green colouring-matter which enables ordinary plants to form their own carbohydrates. Like bacteria they must live, therefore, as saprophytes or as parasites. They form a very large group, probably outnumbering green plants in species, and there is a wealth of morphological difference among them.

Typically, fungi are composed of a thread-like mycelium bearing spores. In some groups a mycelium is lacking and theoretically this absence may be a primitive or a reduced character. Frequently the mycelium is massed together to form a fruit-body often of considerable size. Such structures have not so far been recorded in any of the fungi pathogenic to man (except *Piedraia Hortai*) though occasionally the mycelium becomes compacted into a sclerotium-like mass.

For our purpose we may consider spores as essentially of two kinds, the one formed as a result of a sexual process, or its equivalent, the other being a separation in some way of a part of the mycelium.

CLASSIFICATION.

The classification of fungi is based on the mode of formation of the sexual spore. Three main divisions are recognized, Phycomycetes, Ascomycetes and Basidiomycetes, though there are a few rare genera which do not fit satisfactorily in this grouping ; presumably they are intermediate forms.

Phycomycetes.

As the name suggests, the fungi of this division are algal-like. The lower, presumably primitive forms, are without mycelium, and, moreover, they may have motile sexual spores (gametes) and motile asexual spores (zoospores). There is usually, however, a well-defined stout mycelium,

which, though it may be septate, is in the vegetative parts characteristically continuous. There is a well-marked sexual process, the sexual organs being either differentiated into male (antheridium) and female (oogonium), or, on the other hand, more or less indistinguishable. Differentiated sexual organs occur in the section Oomycetes, undifferentiated, or almost so, in the Zygomycetes. Correlated with this difference in sexuality there is a difference in asexual spore formation. The sporangia in Oomycetes are undivided and on germination give rise to zoospores, though in some genera the zoosporangia are reduced to spores (conidia) which germinate directly. Zygomycetes, in contrast, never possess zoospores; their non-motile spores are produced within sporangia, though here again there is a series leading through a sporangium containing a single spore to a conidium. Oomycetes are commonly parasitic on plants though some are aquatic and may occur on fish (*Saprolegniaceæ*). Zygomycetes, on the other hand, are mostly saprophytic, but a few are considered to be pathogenic to man.

Ascomycetes.

The characteristic of this division is that the sexually formed spores occur within structures known as asci. Eight is the usual number or ascospores, but there are occasional variations from one to many. A constant number of spores within an enclosing membrane is almost a certain sign of an ascus, but the criterion of an ascospore is its method of formation. The spores in the sporangium of a Zygomycete are formed by the dividing of the whole of the contents, whereas ascospores are formed by the cutting out of the cytoplasm around a nucleus, with the result that there is always a portion remaining.

Interesting modifications of the sexual process may be traced in Ascomycetes, for in some there are well-marked antheridia and oogonia; in others, antheridia are absent, while in the majority neither can be found. The mycelium (as in Basidiomycetes) is usually fine and septate.

Basidiomycetes.

This division is characterized by having its spores usually in fours, borne on the outside of a basidium. It includes mushrooms and toadstools, rusts and smuts, and has no importance from the point of view of medical mycology so far as we know, except as toxic agents.

Fungi Imperfecti.

When we consider the fungi concerned in human disease we find that the majority are classed as Fungi Imperfecti. This division is not of the same classificatory importance as the previous ones, but far exceeds them in the number of its species. It receives its name from the fact that the spore-forms known do not include the sexual or perfect stage; they are fungi with an incompletely known life-cycle, or, it may be, with an incomplete life-cycle. So far as concerns us here it is most likely that the majority are the asexual (conidial) stages of Ascomycetes. Many

Ascomycetes are known with both ascospore and conidial stages. Further, many conidial stages are of the same form-genus as those with a known perfect stage.

BIOLOGICAL PHENOMENA.

Homothallism and Heterothallism.

A phenomenon which it is necessary to consider when dealing with Fungi Imperfecti is the possibility of the occurrence of two physiologically different strains. It is now well known that some fungi have mycelium all of the same kind, whereas others have two (or more) different kinds. The phenomenon was first discovered by Blakeslee (1904) in Zygomycetes. The sexual spores (zygospores) of some species have been recorded frequently, whereas those of others were rarely or never found. Much work on the physiology of zygospore-production had been undertaken previously, but no satisfactory explanation had been found. Blakeslee showed that all the spores of a given sporangium might give rise both to sporangia and to zygospores; the mycelia, therefore, are all of the same kind (homothallic). In the simplest examples of the other type the spores from a given sporangium give rise only to sporangia, but when spores from a number of sporangia of different origin are sown on nutrient media zygospores are formed at the junction of some of the resulting mycelia. By pairing mycelia in this way they are found to be of two kinds (heterothallic) each producing only sporangia when alone, but giving zygospores when one of the other kind is present. Mycelia may be indistinguishable except by their behaviour and are usually spoken of as (+) and (−) strains. During the past few years a great amount of investigation has proceeded on these lines and it appears that some form of heterothallism is present in all groups of fungi. It is not yet agreed whether heterothallism is to be regarded as an expression of sexual segregation, for the details are of great complexity and their explanation in terms of sex would probably necessitate a modification of what are usually regarded as sex criteria. However this may be the phenomenon must be taken into account in any attempt to understand the proper systematic relations of the Fungi Imperfecti.

Polymorphism and Pleomorphism.

It is to be noted that there may be more than one imperfect stage in the life-cycle of a given fungus. Everyone who has grown fungi of the ringworm group knows that several spore-forms may be found in a single culture. Here, for purposes of classification, special stress may be laid on one type of spore or the presence or absence of any of them might be made the basis of classification. In many fungi with more than one spore-form, however, we find only the one conidial stage in a culture under a given set of conditions, and only if the conditions are altered does another spore-form appear. It is not infrequent then to find the fungus described under two different names.

We have thus two ways in which a fungus may show more than one spore-form. The known life-cycle may include a perfect and an imperfect

stage ; such a fungus was termed pleomorphic by Tulasne. The other type is where all the spore-forms are conidial. It is preferable to refer to such fungi as polymorphic in contrast to pleomorphic. It is to be noted that polymorphism is frequently used in a loose sense as variation, and that Sabouraud used pleomorphism to indicate a definite change in cultural growth.

Variation.

There is the same variation in fungal species as there is in all organisms. One aspect of this, namely, the response to external conditions, is readily seen in pure cultures on artificial media when startling differences occur with change of media, change of temperature, humidity, amount of light and so on. It follows, therefore, that in cultural work with pathogenic fungi the conditions should be standardized as far as possible.

Strains.

Most species of fungi which have been studied in culture have been found to consist of a number of strains. As in variation, the phenomenon is presumably the same as that in bacteria, but the greater size of the organisms concerned frequently allows a quantitative analysis. Thus strains may be differentiated by their specialized parasitism, by their chemical reactions when grown on culture media or by morphological differences ; occasionally all three criteria may be brought into use.

Saltation (Mutation).

When fungi are grown in culture it not infrequently happens that a part of the growth takes on an appearance different from the rest : the tendency is to term this phenomenon 'saltation' rather than 'mutation' in order to avoid the implication that it is the same as that known as 'mutation' in *Enothera* or *Drosophila*. The occurrence of saltation is frequently related to the composition of the culture medium, and with different fungi different media incite its production. Among the conditions so far investigated are acidity and carbon-nitrogen relation : an excessive preliminary heating of spores also has been shown to stimulate the production of new forms. Most frequently saltation is shown as a sectoring, but it may also occur in patches. Though general attention has been given to this matter only during the last few years, it is to be noted that Hansen recorded its occurrence in yeasts in 1889. Moreover, the well-known 'duvet' formation of the ringworm fungi, well described by Sabouraud, is best regarded as saltation.

The phenomenon of saltation raises many problems. It is to be presumed that it is not necessarily restricted to growth on culture media, and the likelihood of its occurrence in fungi growing on their natural substrata has to be borne in mind. From a practical point of view it is obvious that care must be taken when isolating a fungus directly from a substratum that the original form is obtained.

TECHNIQUE.

Culture Media.

The media in use in mycological laboratories are of the same kind as those used by bacteriologists, but as a rule are devised solely for obtaining good growth and rarely for the differentiation of species ; they have as their basis carbohydrate rather than animal substances. Pathogenic fungi are usually studied in bacteriological laboratories, and consequently it is useful to know the growth appearances on media in common use there. When these prove unsuitable a trial should be made of the ordinary mycological media. The useless modification of media is to be deprecated, for the tracking down of formulæ and the preparation of modified media is a labour which ought not to be demanded for the identification of easily grown species. The best procedure would seem to be to describe the growth on one at least of each group of media, for then both the bacteriologist and the non-medical mycologist would be enabled to make a preliminary diagnosis.*

Examination.

Although it is occasionally possible to identify a fungus from its gross cultural characters it is always necessary to make a microscopic examination. As the method by which the spores arise from the mycelium is of fundamental importance in the identification of fungi, smears and suchlike are of little use. The material should be mounted in some liquid medium. Water or dilute glycerin may be used if the piece to be mounted is first moistened with absolute alcohol, but lactophenol is preferable. If greater clearness of definition is desired this can usually be got by adding a little iodine solution or by dissolving cotton blue in the lactophenol. For cytological work the usual procedure is efficacious.

OCCURRENCE.

Fungi are to be found wherever there is organic material. Many of them are known only as saprophytes, whereas at the other extreme there are some which will grow only on a certain host-plant, a strain of a species of rust, for example, being specialized to a pure line of the host. As intermediates there are species which may live either saprophytically or parasitically. There is considerable support for the view that many fungi pathogenic to man are of this last type.

Saprophytic fungi abound everywhere and, moreover, the atmosphere is loaded with myriads of viable spores. Doubtless many such harmless saprophytes have had pathogenic properties attributed to them. In the examination of fæces one must expect to find saprophytic forms such, for example, as yeasts from the surface of fruits. Spores are usually unaffected by passage through the alimentary canal, and though yeast-like

* Cultures of doubtful organisms should be sent to a laboratory, e.g. the Lister Institute.

fungi are common when fæces are plated out there is often an abundance of moulds when suitable media are used. No importance need be attached to the occurrence of spores (such, for example, as the sculptured spherical spore of the smut *Tilletia*) or even mycelium in fæces unless there is some strong reason for suspecting that an abundance of them may be responsible for the condition of the patient.

POISONOUS FUNGI.

Most toadstools are edible, but a few cause discomfort, and a very few, *Amanita phalloides* in particular, are deadly poison. It is usually possible to identify the fungus concerned by the examination of the spores found in the intestine : these are in abundance, for a mushroom of ordinary size has about two million millions of them.

NOMENCLATURE.

The names to be given to fungi are frequently the source of considerable discussion, and the nomenclature of those causing human disease is in a particularly confused state. Mycologists* subscribe to the International Rules of Botanical Nomenclature (1910). In this, E. M. Fries's *Systema Mycologicum* (1823-32) is taken as the starting-point for the nomenclature of all groups except Uredineæ, Ustilagineæ and Gasteromycetes. A generic name must be used with its original significance, and beginning with the date of the group in the *Systema* the earliest trivial name for a species is to be used no matter whether it is retained in its original genus or transferred to another one. Where the stages of a pleomorphic fungus have received different names that of the perfect stage is to be used. Where a polymorphic fungus with more than one imperfect stage is in question there is no clear ruling except that of priority. It would seem best, however, in medical mycology not to alter the generic name of such a fungus at present unless the second generic name already occurs amongst human parasites ; if the second spore-form needs mention it may be spoken of, say, as the *Hormodendron* form of *Cladosporium*.

The customary method of indicating that a species has been transferred from the genus in which it was originally described is to place the name of the original author within brackets followed by the name of the author who made the transfer.

No attempt has been made here to follow the International Rules, for it is probable that certain names will be conserved during the next few years. The use of the name *Monilia* has been considered by several recent writers, but corresponding difficulties are encountered in the proper application of the names *Torula*, *Oidium*, *Cryptococcus*, *Mycoderma* and *Sporotrichum*. Consequently, rather than take up names recently proposed or show how these fail to achieve their purpose, those commonly used in medical mycology have been adopted.

* Some American mycologists follow the so-called Rochester Code.

Phycomycetes.

Phycomycetes in their typical form differ from Ascomycetes and Basidiomycetes in having a wide, usually more or less continuous mycelium which is rarely (*Endogone*) built up into a fruit-body. For our purpose we may regard the group as including certain genera in which mycelium is rudimentary or absent (Chytridiales).

CHYTRIDIALES.

Chytridiales include a number of forms of great phylogenetic importance. It is becoming generally accepted that fungi were not derived from algæ, and consequently the simple colourless forms are regarded not as reduced because of the adoption of a saprophytic or parasitic habit, but as primitive forms, amongst which we must look both for the origin of more highly developed genera and for the origin of fungi as a whole. There is a great diversity among them and some apparently are of somewhat isolated position. It seems probable that at times it has been a matter of chance whether a given form has been described as a fungus or as a protozoon; on modern views this merely means that different investigators have usually regarded a somewhat anomalous organism as fitting into the group with which they were most familiar. The genus *Rhinosporidium*, which previously was placed in different families of the protozoa, has recently been re-studied by Ashworth, who considers it to be a fungus closely related to *Olpidium*, which is characterized by its lack of mycelium and the production of zoospores (or gametes) within a pyriform or sub-spherical sporangium with or without a well-marked emission tube. It is probable that other organisms pathogenic to man will find their proper classificatory position near to *Rhinosporidium*.

Rhinosporidium.

Ripe sporangium with thick wall except at exit pore, containing numerous spores; trophic stage a multinucleate plasmodium.

Rhinosporidium Sieberi (Wernicke) Ashworth.

The earliest stage seen is about 6μ in diameter with a chitinated envelope, vacuolated cytoplasm, and a vesicular nucleus containing a karyosome. As growth proceeds, protein granules and fat globules appear in the cytoplasm and increase in number and in size. When the cell is about 50μ in diameter the nucleus divides mitotically; this is followed by twelve synchronous divisions resulting in about 4,000 nuclei. Rounded cells are then formed which divide twice to form the young spores, of which there are about 16,000, which may all develop, or up to one-third may remain unchanged. Meanwhile, when the fungus is about 100μ in diameter and has about 128 nuclei the envelope becomes much thickened by the deposition of cellulose on the inner surface except at the point where the future pore will be formed. The mature spore has a chitinous envelope, a vesicular nucleus with a karyosome, and cytoplasm with refringent spherules in the vacuoles: it is spherical or oval, and is

discharged through a pore formed by the rupture of the sporangial wall, where it is unthickened. The ripe sporangium is whitish and usually 0.25 to 3 mm. in diameter.

R. Sieberi usually forms a polypoid growth in the nose, though it has also been recorded from conjunctiva, lachrymal sac, ear and penis. It was formerly thought to be restricted to the male, and, though most cases are reported from India, it has also occurred in Ceylon, U.S.A., Argentine and the Philippines.

Ashworth's reasons for considering the organism to be a fungus are : (1) The nutritive reserves of the trophic phase consist largely of fatty material as in many fungi. (2) Repeated nuclear divisions take place preparatory to spore formation and all nuclei undergo mitosis simultaneously, a feature much more characteristic of lower fungi than of protozoa. (3) Division of cytoplasm occurs without any residual cytoplasm, at a late stage, namely after twelve nuclear divisions, and the cells thus formed undergo two further divisions to form the spores. (4) Between the spores is a mucoid substance, a common feature of the sporangia of fungi. (5) The wall of the sporangium is of cellulose : no undoubted protozoon has a cellulose envelope at any stage. (6) The development of a definite pore for the exit of the spores is paralleled in many lower fungi.

Coccidioides Rixford & Gilchrist.

The characters of this genus are very ill-defined and it is usually considered to be intermediate between *Saccharomyces* and *Monilia*, though the first observers thought it to be a protozoon. The internal spores and the general structure suggest that its proper position is near *Rhinosporidium*, though the occurrence of mycelium in cultures is puzzling.

Coccidioides immitis Stiles.

In tissues, forming abundant spherical cells 3 to 80 μ in diameter, with a very refringent wall 3 to 6 μ thick, sometimes rough or spiny ; filled when mature with rounded bodies 3 to 6 μ in diameter. The spherical cells open in the tissues and allow the escape of the enclosed bodies. No external budding has been seen.

Posadas (1900) distinguishes two kinds of cysts. In the first the contents divide into zoospores (?) which surround themselves with a thick membrane, and, increasing in size, resemble the mother-cyst, which by rupturing sets them free. The development occurs within giant cells and the young parasites are incapable of infecting other cells. This stage is regarded as a vegetative one serving for resistance. In the second kind the contents of the mother-cyst divide up into a very large number of corpuscles, which are at first granular and probably amœboid, then clear and homogeneous. At maturity they are ovoid with sharp outline, 6 to 10 μ in diameter. The mother-cyst ruptures and sets them free ; they penetrate into the surrounding cells and, increasing in size and surrounding themselves by a wall, reproduce the mother-cyst.

These observations have not been confirmed, for all later investigators have dealt with cultures. When sown on culture media the cysts give rise to septate mycelial filaments which form grey translucent colonies raised a little above the substratum but showing a profuse growth below the surface. The abundant production of arthrospores by the mycelium has led to the fungus being placed in the genus *Oidium* or *Mycoderma*. Older colonies are opaque white owing to the formation of aerial hyphæ with chlamydospores isolated or in chains. Later they become brown and show spherical bodies like those seen in tissues. Internal spores have been seen in cysts cultivated anaerobically (MacNeal and Taylor, 1914).

The fungus was first described (Wernicke, 1892) as causing a papillomatous eruption. Coccidean granuloma is fairly common in America, showing close clinical and pathological resemblance to tuberculosis. Mortality is high.

Castellani (1928) has proposed the new temporary genus *Blastomycoides* to include this species, *Blastomyces dermatitis* Gilchrist & Stokes (*Cryptococcus Gilchristi* Vuill.) and *Blastomycoides tutanensis* Cast. The three fungi appear to have little mycological relation.

Da Fonseca (1928) regards *Coccidioides*, *Mycoderma dermatitis*, *Rhinosporidium* and a new genus *Pseudococcidioides* as allied genera belonging to the Protomycetaceæ. The Protomycetaceæ are a family of doubtful and isolated systematic position, but are usually thought to be closely allied to Ascomycetes. The possession of a branching septate mycelium in culture as well as other characters are against Da Fonseca's opinion.

ZYGOMYCETES.

There are two main series, one with sporangiospores (Mucorineæ), the other with conidia (Entomophthorineæ).

MUCORINEÆ.

This series contains several families, but the largest is the Mucoraceæ, which, moreover, includes the genera which are of pathogenic interest. Most species of the family are saprophytic, and viable spores of many of them are regularly to be isolated from the air. All organic substrata are liable to be infected by them; many species have their normal habitat in the soil, where they play a definite part in the breaking down of organic substances.

The mycelium is much branched and is usually continuous, though when old or when fructifications are forming, septa are laid down. When grown in solutions rich in sugar the ends of the filaments become divided up by transverse walls and may divide again, round up and separate off as spores (*oidia*, arthrospores). They may then bud in a yeast-like manner, forming what are known as gemmæ.

Another structure of common occurrence is the *chlamydospore*. Chlamydospores usually arise in any part of a hypha mostly by the cutting out by transverse walls of a portion, which is then surrounded by

a thick resistant membrane. Chlamydospores are very common in some species, and as they are loaded with food reserves and retain the power of germinating for a long time they serve as a means for carrying on during inclement conditions.

All the genera which are of pathogenic importance have globose, pyriform or claviform sporangia containing numerous spores and separated at the base from the sporangiophore by a septum (*columella*) which projects into the sporangium.

It is not possible as a rule to determine the species in their parasitic state. They can be grown easily on various substrata. The medium of most common use is beer-wort agar, but each monographer of the group appears to have his special fancy. The influence of the medium on the growth of these fungi is not, however, so marked as in certain other groups.

Several points require attention in attempting the diagnosis of species, such, for example, as the presence or absence of branching of the sporophore; whether stolons occur and if so their position on the mycelium and their relation to the sporangiophore; the existence of crampons; the dimensions of various parts—the height and width of the sporangiophore, width of mycelium, size and shape of sporangium, columella, zygosporoes, chlamydospores; nature of sporangium wall; whether a portion of the membrane of the burst sporangium remains attached to columella as a collarette.

Mucor Micheli.

Mycelium within or on surface of substratum, at first continuous and colourless, then divided into more or less elongated cells sometimes filled with oil. Sporangiophores upright, simple or branched with definite or indefinite branching. Sporangia spherical, erect or sometimes curved on circinate pedicels, multisporeous, uniform but often of different sizes; in the smallest the membrane may persist, whereas in the large ones the non-cuticularized membrane is encrusted with crystals of calcium oxalate and breaks up almost completely in water (*diffluent*), leaving a collarette at the base of the columella; columella colourless or slightly coloured; spores round or oval, usually smooth, colourless or slightly coloured. Zygosporoes formed on the mycelium, not on special branches. Chlamydospores along the mycelium or in sporangiophores. Oidiosporoes in chains at ends of filaments. Certain species form yeast-like budding cells in sugar solutions.

Mucor is one of the commonest saprophytic fungi and occurs everywhere in laboratories.

Mucor Mucedo Linné.

Sporangiophores erect, forming a tuft up to 15 cm. high, silvery grey, shining, unbranched, 2 to 15 cm. by 30 to 40 μ , aseptate, membrane colourless, smooth, contents colourless or slightly yellowish. Sporangia 100 to 200 μ in diameter, first yellow then dark grey or blackish brown, membrane with needle-like crystals of calcium oxalate, very diffluent,

leaving a collarette ; columella free, cylindrical, campanulate or spherical, 70 to 140 μ by 50 to 80 μ with colourless membrane and contents often red-orange ; spores elliptical or subcylindrical, twice as long as broad, of very different sizes in the sporangium, 6 to 12 μ by 3 to 6 μ , with smooth colourless membrane and slightly yellow or colourless contents. Zygosporos spherical, 90 to 250 μ in diameter ; exospore black, with thick prominent warts, hard and fragile ; endospore colourless with less prominent warts fitting into those of the exospore. On germination the zygosporos gives rise to a sporangiophore. Chlamydospores unknown.

This is the commonest species and from the time of Bennett (1842) has frequently been described as causing disease.

Rhizopus Ehrenberg.

Mycelium of two kinds, one submerged in the substratum, the other aerial, forming stolons with nodes here and there from which rhizoids arise, fixing it to the substratum. At these nodes the sporangiophores occur sometimes singly but usually in clusters. Sporangiophore usually darker and widening above into an apophysis in such a way that the columella is inserted above the point where the spherical swelling is attached to the filament. Sporangia at first white, becoming bluish black, all of one size, spherical or subspherical with flattened base, membrane not cuticularized, uniformly encrusted and entirely diffuent ; no collarette ; columella widely subjacent, hemispherical ; spores round or oval, angular, colourless, bluish or brownish, with cuticularized membrane, smooth or striate, rarely spinulate. Zygosporos naked, forming in the substratum and on the stolons. Suspensor straight, very wide and swollen, without appendages.

Lucet and Costantin (1900) described *Rhizomucor* as a subgenus of *Mucor*. Most writers, however, have treated it as a genus. This is unnecessary, for several species of *Rhizopus* have much the same characters, and although there are slight deviations from the generic description as given here, it is less confusing to regard them as anomalous species than to erect another genus for their reception.

Rhizopus parasiticus (Lucet & Costantin) Lendner.

Tufts, lead-grey or mouse-grey, then greyish brown-yellow. Stolons and rhizoids irregular. Sporangiophores branched, 1 to 2 cm. by 12 to 14 μ , usually in clusters, sometimes in corymbs. Sporangia 35 to 80 μ in diameter, membrane with needles of crystals of calcium oxalate ; columella ovoid, cutinized, slightly brownish, 30 to 70 μ by 24 to 56 μ ; lateral sporangia similar but smaller ; pedicels rarely branched a second time ; spores irregular, smooth, 4 to 2.5 μ . Zygosporos unknown. Grows vigorously on all media, particularly on solid media containing sugar or glycerin. Optimum growth between 38 to 40° C., but can be cultivated up to 53° C. Found in lungs of woman thought to be tuberculous. Pathogenic for rabbit, guinea-pig and fowl (spontaneous) ; intravenous or intraperitoneal inoculation causes death in from three to seven days.

Absidia van Tieghem.

Mycelium forming stolons as in *Rhizopus*. Sporangiphores erect, rarely isolated, usually in clusters of two to five, borne on the curved portion of the stolon and not at the nodes, uniseptate. Sporangia uniform, pyriform, erect, with an infundibuliform apophysis, membrane not cuticularized nor encrusted, diffuent, leaving a short basal collarette; columella darker than the sporangiphore, hemispherical, conical or mammiform, more rarely spinescent or ending in a single long projection; spores round or oval, 5 to 6 μ in diameter, with membrane smooth, rarely echinulate, colourless or bluish. Zygosporangia formed on the stolons, surrounded by circinate cuticularized filaments which arise in a verticil from one of the suspensors. Gametangia straight. On germination, zygosporangium gives rise either to mycelium or to sporangiphores.

Vuillemin (1903) showed that a number of genera which had been recently described were closely allied and could be regarded as comprising the genus *Absidia* sens. lat. He proposed *Lichtheimia* as an additional genus in this series. It is not adopted here, for the characters given are not sufficiently precise, and, moreover, its adoption would obscure the fact that we are dealing with members of a genus which finds its principal habitat in the soil.

Absidia corymbifera (Cohn) Vuillemin.

Sporangiphores decumbent, corymbosely branched, forming a white woolly mat ending in corymbose branches bearing sporangia on somewhat long pedicels; often clusters of branches bearing smaller sporangia occur a little below the terminal corymb. Sporangia erect, colourless, pyriform, with infundibuliform apophysis narrowing gradually up to the sporangiphore, 45 to 60 μ mean diameter (10 to 20 μ up to 70 μ), membrane colourless, transparent, smooth, diffuent, leaving a basal collarette; columella wide, hemispherical or globose, 10 to 20 μ , smooth or with short spines, smoky grey or brownish, as also are the apophysis and pedicel; spores spherical, subspherical or occasionally oval, colourless, 3 μ by 2 μ (rarely 6.5 μ by 4 μ). Zygosporangia unknown. Grows well on beer-wort agar, Sabouraud's agar and sterilized bread. Its optimum temperature is about 36° C. Occurs in soil.

This is the best-known pathogenic species of the group. It has been recorded as causing a generalized mycosis, pulmonary mycosis, and otomycosis. It is pathogenic for animals, particularly rabbit, death following rapidly on intravenous inoculation, more slowly with intraperitoneal.

Ascomycetes.

Few genera of Ascomycetes are concerned in human disease, and consequently it is unnecessary to consider their classification in detail. The forms which are usually regarded as primitive have their asci either without any enveloping filaments (e.g. *Endomyces*) or are surrounded by a loose web of protecting hyphae (e.g. Gymnoascaceae), whereas in the

more typical members there is a well-developed fruit-body of definite shape and size which may be completely closed (e.g. *Perisporiaceæ*), cup-shaped (*Discomycetes*) or flask-shaped (*Pyrenomycetes*).

The arrangement adopted here is one which seems the most convenient though it is not consistent. Yeasts are *Ascomycetes* of a reduced type and reproduce typically by budding. There are a number of fungi which resemble yeasts in everything except that they are not known to produce endospores (ascospores). In attempting a diagnosis they would be looked for among yeasts and it has been thought best to consider them as asporogenic yeasts, though there is no proof that they are such. For convenience, other yeast-like fungi are also classed with them.

The ringworm group is usually placed in *Gymnoascaceæ*, which is a puzzling arrangement. Its members would be searched for in *Fungi Imperfecti* and the fact that their perfect forms are still doubtful makes this their logical position.

Similarly, although the perfect stages of a few species of *Aspergillus* and *Penicillium* are known there are hundreds of which the conidial stages only are seen. These genera, therefore, are here placed in *Fungi Imperfecti* to avoid confusion.

SACCHAROMYCETACEÆ AND YEAST-LIKE FUNGI.

Yeasts are amongst the most widespread of fungi and are common in the soil and in the atmosphere. Their importance in bread-making and in controlled or in uncontrolled production of alcoholic drinks gives them a special place in economic mycology. Except for those employed in commerce, the species for the most part are not well known; those to which pathogenic properties have been attributed are mostly unrecognizable from the descriptions. The precise determination of yeasts is always difficult because of the frequently vague nature of the specific characters; on the other hand, generic differentiation is usually comparatively easy.

Yeasts are unicellular fungi which are round, oval or cylindrical, and reproduce vegetatively by budding.* The daughter-cells usually become free, but when growth is rapid may remain joined in chains. In old cultures, especially in colonies on solid media and in the scum in liquid media, cells often elongate and remain joined on, budding, and so form fairly elongated filaments, a sort of rudimentary mycelium which is sometimes branched and which is capable of giving rise to ordinary buds. This is to be distinguished from the true mycelium with budding cells which is present from the beginning of growth in *Endomyces*, *Monilia*, &c.

Yeasts usually produce alcoholic fermentation of sugars. When deprived of nutrient, particularly after vigorous growth, spores (endospores, ascospores) are formed within some of the cells, usually one to

* The genus *Schizosaccharomyces* is an exception to this as new cells are cut off by a wall; ascus formation is analogous however. *S. hominis* Benecke has been isolated in Germany from epidermomycoses.

four in number but sometimes as many as eight, though there is always a specific tendency towards a fixed number. The endospores usually remain within the ascus until they germinate. The variations in the sexual processes of yeasts are of great interest and are of phylogenetic importance. Often the asci result from the fusion of two equal (isogamous) or unequal cells (heterogamous) which join by a copulation canal; sometimes the ascospores fuse; sometimes there is no fusion of cells or spores.

With rare exceptions the form and dimensions of yeast-cells are not specific, nor are those of the ascospores. It is therefore necessary to make use of physiological characters much as in systematic bacteriology. There are, however, several points of difference, for morphology plays a considerable part. Criteria for the determination of yeasts were worked out principally by Hansen and by Lindner. A month or more is required for precise identification.

The media most used for growing yeasts are beer-wort agar or Difco dried malt extract and gelatin, yeast-water, Raulin's solution, potato and carrot. Examination is usually first made in hanging-drop; if there is contamination by bacteria these can often be removed by adding a little lactic or tartaric acid. It cannot be too strongly stated that sugar reactions alone or with the addition of a description of the lesion are inadequate for a proper identification.

The isolation of the fungus is accomplished by one of the usual methods of obtaining single spore cultures. The presence or absence of a mycelium determines whether the fungus is a yeast (*sensu lato*) or of the *Endomyces-Monilia* type.

The production or not of ascospores is important. This in mycelial forms usually occurs without any special methods being adopted to incite their formation, but in yeasts it is usual only when starvation and inability to bud follow on active growth with an accumulation of food reserves.

The customary methods of obtaining sporulation in a reasonably short time are: (1) The yeast is cultured on beer-wort for twenty-four hours and then recultured for the same time in fresh beer-wort. Some of the fungus is then placed on a cylinder of plaster of Paris half submerged in water in a dish, which is then covered and kept at 25° C. Ascospores form in from one to eight days. (2) After rejuvenating the fungus as in (1) it is sown on Gorodkova's medium. (Peptone, 1 gm.; beef bouillon, 1 gm.; sodium chloride, 0.5 gm.; glucose, 0.25 gm.; agar, 1 gm.; distilled water, 100 gm.). This medium is feebly nutritive. The yeast begins to bud, but this soon ceases and sporulation begins after from one to eight days. Sporulation occurs in most species on potato and carrot, but only after several weeks. A few species which do not spore by (1) and (2) form spores in old cultures on carrot. It is therefore necessary always to try this method where the others are unsuccessful.

In the identification of yeasts the process to be gone through is somewhat as follows: The macroscopic characters on beer-wort at 25° C. are observed at the end of twenty-four hours and then daily. Some species develop exclusively at the bottom of the liquid, forming there a whitish deposit, others grow almost exclusively at the surface, producing a pellicle which may creep up the wall of the containing vessel, forming a ring; usually the pellicle falls to the bottom when the culture is shaken, there forming a deposit, and a new pellicle immediately begins to form. In species with a deposit this may continue, but some species, after from a week to two months, produce a pellicle, with or without a ring, or a ring only, or even fragments of a pellicle. The characters of the pellicle are important. The microscopic characters, general form and dimensions of the cells as seen in a twenty-four hours' culture on beer-wort are to be noted; there is variation, but there is always a dominant type. In old cultures, particularly on solid media, and in pellicles on beer-wort, the cells may elongate and the buds remain joined to form a sort of reduced mycelium which is sometimes branched and can give rise to ordinary cells. This mycelium is sometimes fairly well developed.

The temperature limits and optimum for budding and for pellicle-formation should be ascertained. These vary in an appreciable manner from one species to another. Budding is best observed on beer-wort agar. Similarly, temperature limits and optima and the time required for ascospore formation should be ascertained. This is most conveniently accomplished with Gorodkowa's medium. The mode of germination of the ascospores is important. As usually only a few of these are formed, the presence of vegetative cells complicates matters. A useful method is to place some of the yeast on a slide and heat at 55 to 60° C. for twenty-four hours. This kills the vegetative cells, but does not harm the ascospores. The dry yeast may then be transferred to beer-wort at 25° C.

The fermentation of different sugars is important. It is, however, to be noted that some yeasts never produce fermentation. The sugars usually tried are saccharose, dextrose, lævulose, lactose, maltose, and galactose, mannose and raffinose. Lindner's method for ascertaining fermentation is that in most general use, but it is necessary always to indicate the procedure adopted. The macroscopic characters of giant colonies on agar or gelatin are frequently characteristic.

Similar data should be obtained also in studying asporogenic yeast-like forms. Different authors have proposed additional 'tests', but for diagnosis it is essential to keep them within reasonable limits. It should be realized, however, that special methods of distinguishing between species sometimes greatly reduce the number of necessary manipulations.

Saccharomyces Meyen.

Asci formed directly from vegetative cells without any trace of sexual fusion; spores smooth-walled varying from one to eight in the ascus;

ascus usually opening at the time of germination ; spores germinating by simple budding, sometimes after they have conjugated in pairs. In beer-wort first forming a deposit and if producing a ring or a mucous film only after a time. Giant colony usually shining and humid. Vegetative cells ovoid or globular, ellipsoid or elongated.

The genus is usually divided into six groups according to sugar reactions. The pathogenic species fall into the section where no fermentation occurs or in which it is unknown. Several species have been recorded as pathogenic to man, some causing lesions similar to those of tuberculosis or syphilis.

Debaryomyces Klöcker.

Asci formed after isogamic or heterogamic copulation, normally with a single spore though occasionally having two or three ; spore membrane with small processes. Vegetative cells usually spherical and, like the spores, having a single oil-drop.

About half a dozen species are described as pathogenic to man, usually causing epidermomycoses.

Willia Hansen.

Asci formed without previous copulation ; ascospores, hemispherical with a projecting edge, or lemon-shaped with a central oil drop and a longitudinal bordering band ; ascospores germinating by budding, during which the projecting edge or longitudinal band gradually disappears. In some species the ascospores conjugate before germinating.

W. anomala has been isolated from tubercular sputum.

Cryptococcus Kützing emend. Vuillemin.

Following Vuillemin this generic name is used to designate pathogenic asporogenic yeasts. For the most part the species are of the same type as *Saccharomyces*, behaving similarly in every way, with the exception that they are not known to form ascospores. It is to be noted that the name *Cryptococcus* is not used in its original sense and is adopted here merely to avoid confusion. It is neither logical nor convenient to base a generic distinction on reputed pathogenicity.

In classifying and describing the species of this genus the methods outlined for the study of *Saccharomyces* should be followed.

The species described as belonging to the genus fall into several groups, and premature attempts have been made to emphasize differences by the erection of new genera. De Beurmann and Gougerot (1909) proposed several names on slender foundations, and though these have rarely been adopted there has been more recently a recrudescence of name coining and name changing, which appears to be based neither on proper mycological criteria nor on the strict application of the rules of botanical nomenclature. Ota (1924) separates the described species into two main divisions, those which never produce mycelial filaments and those which

are able to do so ; the second forms a passage to *Monilia*. The species of the first division are grouped into four types :

(1) Cells round, ovoid or elliptic, relatively large, usually solitary or grouped in pairs, with fairly thick membrane and a very clear double contour ; cultures soon ochraceous, becoming greyish with age.

(2) Cells round, ovoid or slightly elongate, usually solitary or grouped in pairs, or sometimes forming chains, membrane not so well defined as in (1) ; cultures usually greyish white.

(3) As in (2), but colonies red or pink.

(4) Cells very small with thin indefinite membrane ; colonies greyish white.

Over thirty species of *Cryptococcus* have been recorded as pathogenic to man. *C. (Torula) histolytica* Stoddard & Culter causes meningo-encephalitis and *C. hominis* Vuill. systemic blastomycosis.

Endomyces Rees.

Mycelium always present, giving rise to yeast-like cells by the budding of its segments and sometimes also to true conidia ; arthrospores are also occasionally produced. Asci formed in cells, resulting from the budding of mycelial segments or sometimes in terminal or intercalary segments of the mycelia, exceptionally in yeast-cells. Ascospores usually few, rarely one or two, usually with a double wall and hemispherical, either with a projecting rim to the flattened surface, with an outer wall of two unequal valves with a projecting line of junction, or with a rough wall and a projecting surrounding band. Ascus formation sometimes preceded by a heterogamous copulation between the terminal segments of two neighbouring filaments or an attempt at isogamous copulation. Germination of ascospore results in direct formation of mycelium or in the production of buds.

Endomyces capsulatus Rewbridge, Dodge & Ayers.

Mycelium in lesions consisting exclusively of budding cells. Easily cultivated, growing best on media with low hydrogen-ion concentration and complex nitrogenous compounds. When first isolated and in acid media, mycelium consists of coarse thin-walled hyphæ, 3.5 to 4 μ in diameter, composed of nearly isodiametric cells ; hyphæ often somewhat contorted suggesting those of *Madurella*. On neutral media with protein as the principal source of nitrogen the hyphæ are slender, branched, 1.4 to 1.5 μ in diameter with long, mostly uninucleate cells. Budding cells about 3.5 by 5 μ are abundant on acid media but absent on neutral media. Chlamydospores terminal on short branches or in chains : they develop from a mycelium resembling the raquet mycelium of *Trichophyton* with cells 6.5 to 7 μ in diameter at the swollen end and 2.6 to 3 μ in diameter at the other end. On corn-meal agar large spherical cells, 11.3 μ in diameter, develop at the end of short branches ; they have very thick walls but their contents seem to be degenerating. Conidia sessile, pyriform, 5.5 by 6 μ , developing irregularly along the mycelium but

usually near a septum, more abundant on neutral media with protein nitrogen. Endospores (endoconidia, globules internes), ellipsoidal, 2.6 by 3.2 μ on Sabouraud's dextrose agar, formed by rounding up of the protoplasts into deeply staining ellipsoidal masses; no spore-wall observed. Sexual reproduction, when it occurs, heterogamous; cell of filament swells and fuses with an apparently little differentiated cell on an adjacent hypha. Asci terminal on long filaments, eight-spored. Ascospores hyaline, spherical, smooth, 2 μ in diameter, observed only in cultures on Sabouraud's dextrose agar about nineteen weeks old.

Clinical records and autopsy findings in systemic infection; organism isolated from tissues. Pathogenic to mice.

Monilia Auct.

The application of the generic name *Monilia* is a matter about which much has been written. Mycologists use the name for the conidial stage of the Discomycete, *Sclerotinia*, usually parasitic on plants, where the conidiophores are erect, the cultures are usually downy and there is as a rule no fermentation of sugars. The name is used here in the medical sense and the genus is characterized as having sparse mycelium readily dissociating, reproducing by blastospores, giving white, usually glabrous colonies on solid media; the blastospores form long, relatively stable chains usually inserted apically but sometimes laterally on the creeping septate hyphae which rarely branch.

The genus is unique in the number of species which have been proposed purely on biochemical grounds. It is said to be impossible to distinguish between several species causing thrush, some types of bronchomycoses and some dermatomycoses on purely morphological lines, and consequently Castellani has introduced a biochemical classification based on the methods used in classifying dysentery bacilli. By differentiating between the fermentation of different sugars, twelve groups are distinguished and upwards of forty species. The problem is reduced to its simplest terms in such a diagnosis as '*Monilia pseudo-Guilliermondii* Castellani, 1916. Clots milk'. The main criticisms of such a classification are: (1) the inconsistency of the reaction, and (2) the needless multiplicity of species. There is some difference of opinion about the first, but all are agreed that the fermentative reaction of some species varies with age, and Castellani states that the method can be applied with safety only on the original isolation. The second point resolves itself into considering whether specific distinction is reasonable, even when reactions are certain. There can be no doubt that when used in a precise manner the information about sugar reactions is very valuable, but for the mycologist it is of secondary importance. Apparently no real effort has been made to separate many of the so-called species on purely morphological and cultural grounds. Those I have had in culture seem to be amenable to ordinary mycological procedure, and, though a satisfactory classification is admittedly difficult of attainment, specific differentiation is a far simpler matter here than in yeasts.

Monilia albicans (Robin) Zopf.

The fungus shows almost the same characters *in situ* and in culture, a septate branched mycelium which is never highly developed, and yeast-forms, arising from the mycelium by budding. The relative amount of mycelium and 'yeast' depends upon conditions, one sometimes predominating entirely. Thus in Raulin's solution there is practically no mycelium, whereas it is abundant on carrot. The cells of the mycelium are uninucleate. The yeast-cells are spherical, oval or elongate and vary in size: in Raulin's solution they are fairly large and spherical. At the end of certain filaments chlamydospore-like structures develop, 10 to 20 μ in diameter, filled with glycogen and surrounded by a thick three-layered wall. When placed on fresh media the chlamydospores give rise to mycelium and yeasts. Smaller yeast-like internal structures are sometimes formed within the mycelial cells. Vuillemin considers these to be resting stages also, but as Guilliermond has pointed out, they are probably formed in cells with poor contents by the intrusion of buds from an adjacent cell.

Growth occurs between 20 and 39° C., both on solid and liquid slightly acid media. In liquid media growth is always slow and a film is never formed; usually only a floccose, or powdery slight deposit is seen. On solid media growth is more abundant and more rapid. Gelatin is not liquefied readily. The macroscopic appearance of the culture on agar depends on the composition of the medium. On Sabouraud's ordinary medium, the colony is whitish, humid, and has a crinkled surface.

The fungus is the principal agent causing 'thrush'. It may spread to the oesophagus, stomach and intestine. I isolated it on three occasions from fæces in Macedonia.

There appear to be several strains of the fungus, but with our present imperfect knowledge it is impossible to decide whether some of these, and which, should not best be treated as distinct species. Vuillemin (1898) found asci in old cultures on beet, but did not determine the conditions of their formation. They were large, ovoid or ellipsoid cells, 4 to 5 μ in diameter, formed by a lateral or terminal budding of the segments of the mycelium or sometimes by germinating chlamydospores. They had a thick wall and contained four flattened, slightly reniform ascospores, 1.2 to 3.5 μ by 1.4 to 1.75 μ , also with thick walls. Germination was not observed. In consequence, Vuillemin gave the name *Endomyces albicans* to the fungus. During the past thirty years many attempts have been made to confirm Vuillemin's observations but without success. Landrieu (1912) has proposed to designate this exceptional form by the name *Endomyces Vuillemini*.

Monilia psilosis Ashford (*Parasaccharomyces Ashfordi* Anderson).

Cells round or slightly oval in young cultures, 4.5 by 5 μ , becoming also elongated, elliptical or irregular in old cultures; giant cells common. Septate mycelium 2 to 5 μ in diameter, develops in gelatin hanging-drop

and in old cultures. Budding occurs from any point on the young cells, but usually near the ends of segments in old cultures. On glucose-agar the streak is filiform, raised, glistening, chalk-white and smooth; later the central portion may become rugose or pitted; the edge of the streak may remain entire or may become decidedly filamentous, due to the outward-growing hyphal elements under the surface of the medium. Growth in gelatin stab at first filiform, later developing scattered bushy clusters of filaments. In liquid sugar media and beer-wort a very evident ring formation occurs; no pellicle is present.

Glucose, maltose and l  vulose are fermented and occasionally sucrose and galactose. Yeast-water sugar media become more alkaline. Litmus milk is rendered alkaline in two weeks but is not clotted. Gelatin is rarely liquefied.

This fungus is frequently regarded as the cause of sprue. It is very close to *M. albicans* and some investigators have not distinguished between them. Castellani lists seven species of *Monilia* recorded for sprue, and considers *M. psilosis* as probably synonymous with *M. enterica* Cast., the description of which reads 'found in stools by Castellani. Milk is rendered alkaline. Gelatin and serum are not liquefied. For sugar reactions see table'.

Enanthiothamnus Pinoy.

This genus is very closely related to *Monilia*, the chief distinction being that the spores are borne in whorls round the upper walls of the mycelial segments.

Enanthiothamnus Braultii Pinoy.

Mycelium branched, creeping, 2 to 2.5   wide, regularly septate, easily breaking apart at the septa; conidia simple, colourless, arising directly on the filaments, 2 to 2.5   by 1 to 1.5  , arranged in verticils in the upper part of the cell segments at the level of the septum. The fungus grows readily on all the usual media from room temperature up to 37   C., and gives yeast-like colonies which are waxy, white, mamillate, irregular, with a slightly yellow centre. In liquid media a pellicle is formed.

Isolated from three tumours resembling large molluscum contagiosum but more coloured and bulged; from an Arab's buttock. The mycelium in the tumours showed as septate filaments, 1.4   wide, with swellings 2   wide along their length or at the ends. Pathogenic for guinea-pig by intraperitoneal injection and for the rat by subcutaneous injection.

PLECTASCINE  .

This group, which is variously interpreted by different writers, includes two families which are of medical interest, Gymnoascace   and Aspergillace  . It is characterized by the possession of closed fruit-bodies (perithecia) within which the ascogenous hyph   branch irregularly and produce asci which are usually globose and are without definite

arrangement. Gymnoascaceæ have a loose envelopment of hyphæ to the perithecia. It is to this family that most authors relate the ringworm group as imperfect stages. Aspergillaceæ have a loose pseudo-parenchymatous wall to the perithecium. The genera *Penicillium*, *Aspergillus* and *Allescheria* are important as human pathogens. The first two are known principally in the conidial stages and are considered later among the Fungi Imperfecti. The three genera are distinguished solely by the characters of the imperfect stage.

Allescheria Saccardo & Sydow (*Eurotiopsis* Cost.).

Conidia in chains borne on loose branched hyphæ on lateral as well as terminal short branches or sporophores. Perithecia small, spherical with thin membranous wall. Asci globose or subglobose, with eight ovate spores.

Allescheria Boydii Shear.

Perithecia numerous, crowded, covering the surface of the medium, usually erumpent or sub-superficial, globose, thin, membranous, dark brown, astomate, 100 to 200 μ in diameter; asci globose or subglobose, thin-walled, evanescent at maturity, 10 to 20 μ in diameter; paraphyses none; ascospores eight, globose to subglobose or somewhat ovoid, continuous, smooth, pale yellowish brown when mature, globose form about 7 μ in diameter, the other mostly 5.5 to 7 μ by 4 to 4.5 μ .

Byssoid conidial form (*Cephalosporium Boydii*), thin, floccose, white at first, soon grey, margin radiate-fimbriate, later changing to pale greenish ochraceous as sporulation begins, fertile hyphæ much branched, spreading; conidiophores lateral or terminal, mostly short; conidia adhering in small or large subglobose masses, continuous, subglobose to oblong-elliptical, very variable in size and shape, hyaline at first, becoming pale, yellowish brown when old, smooth, 8 to 15 μ by 4 to 7.5 μ , mostly 10 to 12 μ by 5 to 6 μ .

Coremia (*Dendrostilbella Boydii*) with dark brown synnema very variable in height and thickness, 200 to 300 μ or more high, head subglobose; sporophores alternately branching, ultimate branches once or twice the length of the conidia; conidia practically the same size, shape and colour as in the byssoid condition and adhering in a globular mass after abstriction.

The fungus grew well on corn-meal agar or glycerin-agar, and in a fortnight produced all the spore-forms, beginning with conidia, which were regularly followed by perithecia on or under the surface.

It was isolated from a lesion in a human ankle at Texas. The injury was caused twelve years previously by penetration of a thorn in the sole of a foot. After apparently healing, swelling occurred which became soft and finally ruptured, discharging bloody pus. This was repeated at intervals. Granules were found in the diseased tissue, and these, when crushed or sectioned, showed fungal mycelium apparently in pure culture. Inoculation experiments with guinea-pigs were unsuccessful.

ASTERINEÆ.

Asterineæ are a group of fungi which were formerly included in Pyrenomycetes but in recent years have been classed separately in Hemisphæriales. They have a disc-shaped fructification with a scutellate cap of radiate structure which ruptures irregularly as there is no opening (ostiole). Hemisphæriales are probably a biological rather than a systematic group, parasitism having imposed upon them considerable superficial resemblances. Asterineæ are mostly leaf parasites in tropical regions which are only now being explored mycologically. What has been called the 'climat Astérineen' is where there is more than one metre of rain annually. It has been pointed out by Langeron (1929³) that the morphological characters and geographical distribution of *Piedra* are analogous to those of Asterineæ.

Piedraia Hortai (Brumpt) Da Fonseca & Leao. *Trichosporum* Brumpt.

This fungus causes *pie-dra*, a disease of the hair which shows itself as hard, resistant, dark nodules of various sizes, some being visible only under the microscope. Fungal mycelium can readily be seen in the smaller nodules, but the larger ones require first to be treated with potash or some other clearing agent. The nodule is in the form of a stroma, that is a compact, tissue-like mass of interwoven fungal hyphæ, which here are bound together with a greenish yellow viscous substance. Distinct, radiating, dichotomizing hyphæ occur at the periphery forming a lighter-coloured fringe. The mass of the stroma contains large numbers of isolated oval asci in which there are usually eight spores, though occasionally there may be six or twelve. The ascospores are fusiform, slightly curved, with a cilium at each end; occasionally they become septate. The ascus appears to arise at the expense of a cell which increases in size. No ostiole has been observed in the fruit-body, but the ascospores are readily set free in water by the gelatinization of the surrounding cells. The ascospore on germination gives rise to a septate mycelium. The fungus can be cultivated on Sabouraud's maltose medium. The colonies show the same general structure as does the fungus on the hair, except that the stroma is sterile, no asci being formed. The colour of the colony varies with age and with the amount of light.

Piedraia is close to the genus *Protothyrium*, which is a weak external parasite on living leaves on *Salvadora persica*. Like *Trichosporum* and unlike *Trichophyton* and its allies, it behaves more as an epiphyte than as a parasite, its nutriment being obtained mostly from the exterior; the surface of the hair is hardly raised or dissociated at the point of attachment of the fungus, adherence to the hair being due to the marginal fringe of the asco-stroma. The morphology of its allies suggests that a close study of the mode of attachment of *Piedraia* might be of interest.

The ready emergence of the ascospores in contact with water (and possibly sweat) explains how the disease spreads generally, whereas family and school epidemics are due to contact.

It seems probable that *Trichosporum giganteum* Behrend, which causes piedra in Columbia, will also prove to be a *Piedraia*, for so long ago as 1878 Desenne gave an account which suggests the presence of asci in this fungus.*

Fungi Imperfecti.

From a theoretical point of view this is a purely artificial division of fungi, comprising as it does merely conidial or imperfect stages. In practice, however, Fungi Imperfecti play a large part in both animal and plant pathology. Academically they are of little interest and general text-books devote very few pages to them, with the consequence that it is difficult for a non-mycologist to obtain a clear idea of their significance and classification. Originally, every stage of a fungus was described as an entity and the several forms received different generic and trivial names. When the stages of a life-cycle are joined up, these names may continue to be used, and as it is not usual to give a binary name to the conidial stages discovered in the working out of the life-history of a known perfect stage, or vice versa, a somewhat puzzling difference in nomenclature has arisen.

Obviously, a species comprises all the stages in its life-cycle whether there is no imperfect stage or one or more. It must be understood, therefore, that when genera and species are spoken of in Fungi Imperfecti there is not the same significance to be attached to them. Many mycologists therefore speak of form-genera and form-species. The method of reproduction is purely vegetative and this has to be kept in mind when considering the changes which they often undergo in culture.

Fungi Imperfecti far outnumber the rest of the fungi. It is necessary to classify them in order to identify them and it is obvious that this arrangement must be purely artificial. The matter is complicated by the fact that in addition to two or more imperfect forms occurring in a life-cycle, the same form-genus occasionally is found as the conidial stage of two widely separated fungi and the same genus has not always the same form-genus as its conidial stage. Saccardo's system (1884) is the only complete one. He arranged Fungi Imperfecti in three main groups: Sphærosideæ with spores formed within flask-shaped structures (pycnidia), Melanconieæ with spores in an immersed cavity which has a wall formed by plant tissues or other substratum, and Hyphomycetes with spores borne directly on a mycelium, which may be simple or in bundles or cushions. Only the last group is represented in human disease.

The Hyphomycetes are divided into four main families: in the first two the mycelium is separate, in the others compacted.

* 'Les parties centrales de ces nodosités, vues sur une de ces coupes transversales, sont formées par un stroma cellulaire, semblable à celui qui recouvre leur périphérie et dans lequel on trouve quelques cavités en forme de conceptacles, contenant une ou plusieurs grosses cellules incolores qui sembleraient être alors des thèques (?)'.

Mycelium not compacted :

Hyphæ colourless or brightly coloured .. Mucedinaceæ.

Hyphæ dark coloured or black Dematiaceæ.

Mycelium compacted :

Conidiophores in compact fascicles .. Stilbaceæ.

Hyphæ and conidiophores forming a globose or discoid pad which is usually waxy or subgelatinous Tuberculariaceæ.

The families are divided according to the type of spore, *hyalo*—signifying colourless spores and *phæo*—dark coloured spores. Following this out for the Mucedinaceæ we have :

Undivided spores Hyalosporææ.

2-celled spores Hyalodidymæ.

3- or more celled spores Hyalophragmeæ.

Spirally curved septate spores Hyalohelicosporææ.

Forked or stellate septate spores .. Hyalostaurosporææ.

Each of these is then subdivided, e.g. Hyalosporææ :

Micronemeæ—conidiophores and vegetative mycelium more or less indistinguishable.

Macronemeæ—conidiophores readily distinguished from the vegetative mycelia.

Macronemeæ is then divided up according to the way in which the spores are borne, in heads, irregularly, or on verticillate branches, and so on.

This system is the one followed in all purely mycological works, mainly for the reason that it is fully worked out together with the generic and specific descriptions of all known forms in the *Sylloge Fungorum* (1884–1927). It is not satisfactory, however, because frequently the diagnostic characters are not so clearly marked as such an arbitrary system demands, and, moreover, the presence or absence of a minor character often widely divorces what are apparently closely related forms.

There have been few attempts to formulate a more rational classification. Vuillemin, however, has proposed one in outline for Hyphomycetes which has been adopted by most recent writers on medical mycology. This shows so many advantages over Saccardo's system that it would doubtless be used by mycologists generally if the details were worked out. It might almost be said that the changes in the older system were necessitated by the introduction of the pure culture methods of bacteriology into the study of mycology. Previously the discovery of a sporophore of a certain type was all that was necessary to name a fungus and the presence of additional structures was usually ignored.

Most modern mycologists having broken away from the unwieldy terminology coined by the older workers tend to lump all reproductive bodies under the general term 'spore', using the term 'conidium' if wishing specially to refer to that of an accessory form. Vuillemin (1910) has shown that the 'spores' or 'conidia' of Hyphomycetes are not all

of the same type and that this can be made the basis of classification. His views may be summarized in considering his classification as most recently modified. So early as 1904 he separated off *Actinomyces* and its allies from all other fungi, grouping them as Microsiphonæ: these do not here further concern us. Typical Hyphomycetes are divided into Thallosporeæ, Hemisporeæ and Conidiosporeæ, depending upon the kind of spore produced. Taking them in the reverse order for ease of explanation, we find that the Conidiosporeæ possess spores clearly differentiated from the mycelium from their initiation; these are true conidia: the Hemisporeæ have spores which are not so clearly marked off, as the fertile mycelial filament, instead of differentiating, continues to grow and finally divides up into a series of segments which function as spores: the Thallosporeæ have spores which are produced by the budding or dividing up of the mycelium.

It cannot be held that finality has been achieved in classifying the Hyphomycetes, and various modifications will doubtless be introduced on the lines suggested by Vuillemin's system, which, however, as it stands, gives a clearer idea of essential characters than does any other attempt. So as not to complicate the presentation unduly, further details are given under the different groups.

HYPHOMYCETES.

THALLOSPOREÆ.

Spores (thallospores) formed by the budding, segmenting, or thickening of the mycelium, forming respectively blastospores, arthrospores or aleuriopores.

BLASTOSPOREÆ.

Spores (blastospores) produced by budding as in yeasts, the budding occurring before and after liberation. By definition the group includes genera such as *Monilia*, which have been treated for convenience as asporogenic yeasts. Spore increase by budding is not confined to this group, but is found also in Mucorineæ, Ustilagineæ, and, more rarely, elsewhere, but in these budding is a secondary process. *Hormodendron* Bonord., *Cladosporium* Link and *Malassezia* Baill. are genera of Blastosporeæ to which pathogenic species are attributed.

Phialophora Medlar.

Mycelium of brown, septate, cylindrical hyphæ which show a tendency to cohere in rope-like strands, the ultimate branches and branchlets tend to become moniliform. Aerial conidia produced by specialized sporogenous cells which arise terminally or laterally from the branches; abjoined at maturity, simple, formed through successive proliferation into the cup-like termination of the sporogenous cell, to which they cohere in a globose, gelatinous mass. Sclerotic cells and spores in *Monilia*-like chains are also produced in the substratum.

Phialophora verrucosa Medlar.

Sporogenous cells, short, ampulliform or more elongate, usually terminal or irregularly distributed near the ends of the ultimate branchlets, the lips of the terminal cups spreading ; spores ovoid to ellipsoid, somewhat variable in form and size, usually about 4 to 5 μ by 2 to 3 μ ; hyphae 2 to 6 μ in diameter.

This fungus was first isolated from a chronic skin lesion on a man's buttock. It grows on all ordinary laboratory media as a black, felt-like mycelium with faintly granular protoplasm containing fat droplets of varying size : the cells are uninucleate. Sclerotic cells are formed under conditions unfavourable to normal growth and also in tissues. They may undergo septation in more than one plane and so form sclerotium-like cell masses. The fungus is pathogenic to rats and mice, producing lesions similar to those in man.

Blastocystis hominis Brumpt.

Blastocystis hominis Brumpt is now usually considered to be a fungus. The recording of endogenous spore formation by Lynch, Aragus and Knowles and Gupta suggests that its proper position is not in Blastosporeæ but either among the true yeasts or near to *Rhinosporidium*. The details of spore formation are not sufficiently precise for placing this anomalous genus.

ARTHROSPOREÆ.

(Vuillemin includes *Actinomyces* and its allies in the group as Nocardiaceæ, the forms with typical hyphæ being classed as Mycodermaceæ.) Spores of two types, arthrospores and aleuriospores. Arthrospores are formed by the dividing up of the mycelium by transverse walls into a number of closely packed segments which round up and become free, either remaining thin-walled or developing a thick-wall and reserve food-materials to form chlamydospores. Arthrospores include the ' oidia ' of most mycologists. Aleuriospores resemble conidia in form, structure, dimensions, position, colour, constancy and manner in which they are borne. They are really chlamydospores in that at first they form a part of the mycelium and that in spite of their differentiation in development they are not liberated until the mycelium ruptures or collapses.

It should be noted that the division into Blastosporeæ and Arthrosporeæ is not absolute, for both types of spore production occur in some genera. Such genera are here placed in Arthrosporeæ.

Oidium Auct.

Mycelium hyaline or light coloured, septate, disarticulating into arthrospores with square ends, which subsequently become rounded. Forming a pellicle when grown in liquid media and humid yeast-like giant colonies on solid media.

The name *Mycoderma* has been used for this genus by Vuillemin and Jannin, and *Geotrichum* by Laubière. The precise significance to be given to the three names can no longer be regarded simply as a matter of the application of rules of nomenclature, but as one for international agreement. The genus as understood here differs from the *Oidium* of plant pathology, which is the conidial stage of Erysiphaceæ; no species of this last genus has been grown *in vitro*.

Only about half a dozen species are regarded as pathogenic to man; several other pathogenic fungi are, however, usually listed as species of *Oidium* or *Mycoderma*.

Oidium pulmoneum Bennett.

Mycelium filiform, 4 to 10 μ in diameter, soon breaking up into segments 30 to 50 μ long, the segments then dividing up into quadrangular arthrospores 6 to 12 μ by 4 to 5 μ , which sometimes form terminal chains; arthrospores, when free, often rounding up and varying in diameter from 4 to 24 μ . Forming a pellicle in liquid media and white, reticulate, slightly raised colonies on solid media.

Usual in the intestines and the buccal cavity, being frequently observed in sputum; sometimes producing lesions clinically identical with blastomycosis. In lesions occurring as ovoid cells, averaging 7 by 5 μ , or as short filaments 9 to 17 μ by 2 to 3 μ , sometimes placed end to end.

Trichosporum (Trichosporon) Behrend.

Mycelial segments rounded or polyhedral by reciprocal pressure, forming brown, shining, irregular thickenings on hair; more or less superficial, but the cuticle is affected and partly separated from the rest of the hair. Sections of the mass usually show flattened, thin-walled cells at the base, then more or less isodiametric cells, and above these a series of thick-walled cells regularly superposed.

According to Ota (1926) the cultural characters of the species assigned to this genus (except *T. giganteum* and *T. Hortai*, see p. 33) show very close affinity. In culture, either on solid or in liquid media, they possess yeast-forms (sometimes with pseudo-conidia), oidial forms, mycelia and chlamydospores. He provisionally regards *Mycoderma cutaneum*, *Parendomyces asteroides*, *P. Balzeri* and *Hemispora rugosa* as belonging to this genus.

The yeast-form is the principal stage in culture. The cells are spherical or more frequently ellipsoid. The opening between the parent cell and the budding cell is not always narrow, and as sometimes it remains without a cross wall a linear chain may arise much as in *Monilia*. The bud frequently forms obliquely to the long axis, and as the next bud repeats this at the opposite angle a zigzag arrangement results, which, if repeated in secondary chains, gives a much-branched appearance.

The oidial forms arise either by the segmentation of the mycelium or of a yeast-cell.

Pseudo-conidia occur as lateral buds on the mycelium and may be attached to a tooth. Mycelium may develop from an initial spore or from one of a chain of blastospores. It often branches by the swelling and growing out of the proximal cell which pushes the terminal cell to one side and continues in the main direction of growth. This may be repeated by a branch of the third order. The mycelium is of short duration, soon segmenting to form arthrospores.

Torula Persoon emend. Sacc.

Sterile hyphæ absent or present, and downy, branched, septate, hyaline or fuliginous. Conidiophores absent or represented by short, lateral branches. Reproductive elements formed by the disarticulation of the whole filament (arthrospores) or arising directly from the filaments, which bud in their turn to form new colonies, or as chains of true conidia which later dissociate more or less completely. All spore-forms are dark coloured, black, brown, olive-brown or grey, variable in shape, rounded, elliptical, ovoid or almost fusiform, smooth, rugose, or sometimes verrucose.

The generic name *Torula* is ambiguous. It is used here in the usual mycological sense though only because the fungus described below was attributed to it. In medical works it is more frequently used for non-pathogenic asporogenic yeasts. (*Torulopsis* Berlese.)

Torula Jeanselmei Langeron.

Mycelium well developed, filamentous, often coremiolate, fuliginous. Hyphæ branched, septate, formed of keg-shaped segments at the base, torulose, 5 to 5.5μ in diameter, and higher up of elongated and narrow segments, 2 to 2.5μ in diameter. Blastospores rounded or elongated, very caducous, lateral or terminal, and then in clusters at the ends of lateral branches: the blastospores bud rapidly, sometimes even before their fall, and give rise to new colonies. Arthrospores formed by disarticulation of the torulose elements at the base of the mycelial filament.

The fungus was isolated from a swelling on the foot of a mulatress of Martinique. The lesion formed a blackish brown irregular mass, elastic in consistency, but fairly hard and covered with several very soft papulæ with a thin integument. Punctures gave yellow pus containing soft black grains the size of a pinhead. These grains were composed of twisted worm-like mycelium and spores, and readily gave cultures on all the usual media.

Madurella Brumpt emend. Pinoy.

Mycelium septate, reproducing by fragmentation; arthrospores formed secondarily by binary division of the segments. Growing well at 37°C . The species of this genus cause black mycetomas in man.

Madurella mycetomi (Laveran) Brumpt.

Mycelium greyish white, becoming yellowish with age and blackening media containing sugar. Arthrospores variable in size, 2 to 5μ in diameter.

Sclerotia black, sterile, 0.5 to 1 mm. in diameter, formed within the culture medium. Invading the skin, bones, muscles and connective tissue of man, giving rise to mycetomas with black grains which are small, hard, rounded, and more or less warty, morphologically resembling the grains formed in culture. Very widely spread in Africa.

Indiella Brumpt.

Mycelium white, parasitic in bone, muscles and connective tissue, varying from 1 to 5μ and 10μ in diameter, septate, branching laterally from time to time, never secreting pigment, uniting to form grains sometimes comparable to sclerotia, and which characterize the different species. Numerous chlamydospores, usually terminal, occur within the grains. The species of this provisional genus cause white mycetomas in man. They have not been cultivated.

Indiella Mansoni Brumpt.

Mycelium white, fairly slender when young, 1 to 2μ in diameter, with septa 15 to 20μ apart, becoming irregular with age, 3 to 5μ in diameter, with septa only 5 to 10μ apart, showing a large number of chlamydospores, usually terminal, rarely intercalary, 5 to 12μ in diameter, usually spherical and undivided, rarely segmented. Grains very small, flattened, 200 to 250μ in diameter. Known from India and Central China.

Glenospora Vuillemin.

Hyphæ septate, branched. Conidia (aleuriospores) attached to the hyphæ for a long time, large and smooth, oval, truncate at the base, sometimes stalked, arising terminally or laterally. A very polymorphic genus showing *Trichothecium*-, *Verticillium*- and *Cephalothecium*-forms. Vuillemin regarded this genus as the same as *Glenospora* Berk. and Desm., an American genus of plant parasites. I have thought it preferable to indicate my dissent from this view by attributing the genus to Vuillemin rather than by bestowing a new name.

Glenospora khartoumensis Chalmers & Archibald.

Hyphæ septate, branched, pale when young, 1.4 to 2.8μ in diameter, when very old reaching 4.2μ and becoming tinged greenish black, and having interstitial black pigment; segments varying in length, average about 12.5μ . Clear or dark-coloured thick-walled chlamydospores, 14 by 11.3μ , occur in old cultures, but are especially marked in black masses on surface growths on potato infusion and nutrient gelatin. Spores truncate at the base, pale, 4 to 5μ by 3 to 4μ , borne at the ends of hyphæ, on stalks which look like sporophores, or acropleurogenously on the main hyphæ with or without short stalks; spore-bearing hyphæ usually branched and often segmented below, where the spore produces one, or more rarely two, lateral projections, giving an appearance as if of one central and two lateral spores. Grains consisting of central light portions surrounded by thick radially striated cortex; centre composed of segmented branched hyphæ and roundish, oval or irregular chlamydospores,

embedded in a very hard sepia-coloured matrix ; cortex formed of more or less regularly arranged radial hyphæ and some chlamydospores also embedded in a similar homogeneous matrix.

Causing a black maduromycosis in the foot of a native boy in the Sudan.

Scedosporium Saccardo.

Aleuriospores colourless or bright coloured, borne on the decumbent conidiophores, which are oppositely or alternately branched.

Scedosporium apiospermum Saccardo.

Mycelium creeping, filiform, interwoven. Conidiophores decumbent, sparsely branched, rarely septate, 2.5 to 3 μ in diameter, hyaline, with ascending branches slightly narrowed above, bearing a single spore ; aleuriospores pyriform, oblong, sometimes obovate, narrowed and truncate at the base, 14 by 5.6 μ or 11 by 5.7 μ , more rarely globose, smooth, often a little contracted below the middle, hyaline, then a dirty yellowish pink. On potato the colonies are white then somewhat blackish, somewhat tufted, 3 to 5 mm. in diameter.

Causing a mycetoma of the foot in Italy, Brazil and Algeria. It forms white grains 100 to 1,000 μ in diameter, composed of septate filaments 4 to 5 μ in diameter, and of large ovoid spores 12 to 14 μ by 6 to 7 μ .

Scedosporium sclerotile (Peper) Brumpt.

Causes a black maduromycosis ; recorded from Sardinia.

THE RINGWORM GROUP.

This group is mentioned here to indicate its systematic position, but on account of its medical importance it calls for more detailed treatment than has been given to the parallel groups, and a separate section has therefore been devoted to it (see p. 46).

HEMISPOREÆ.

This section is represented by the genus *Hemispora*.

Hemispora Vuillemin.

Mycelium abundant, hyaline, narrow, septate, branched. Fertile hyphæ branched at the base. Each conidiophore-branch ends with a vesicle (protoconidium) formed from an annular contraction by a thick, brown, rigid wall ; the vesicle becomes converted wholly or in part into a series of spore-like segments ; sometimes it elongates into a new conidiophore or puts out branches which behave in the same way.

H. stellata Vuillemin.

Mycelium compacted into sessile white discs, 0.5 to 2.5 mm. in diameter, covered with conidiophores which form a ferruginous brown stellate elevation. In culture forming irregular cerebriform layers of indefinite extent. Mycelium 2 to 3 μ wide. Hemisporae 4 to 8 μ , subspherical, 2.6 to 3.5 μ , with a fuliginous membrane, granular except on the detached face, sometimes more extended and barrel-shaped.

The fungus is a common saprophyte and occurs fairly frequently in the air of laboratories. Easily cultivated on all the usual media. According to Guéguen as many as 130 hemisporos may occur in a chain. It was first recorded as a parasite of man by Gougerot and Caraven (1909). It has not been seen in lesions except in onychomycosis, but has been isolated several times. Hemisporosis is very similar to sporotrichosis, and diagnosis is confirmed in the same way—sero-agglutination, coagglutination and fixation of complement.

CONIDIOSPOREÆ.

The method by which the conidia are borne gives the basis for the subdivision of the group. They may arise directly on the mycelium ; Sporotricheæ : be borne on a special branch, the sporophore ; Sporophoreæ : or may be borne on a more specialized flask-shaped structure separated off by a wall and with a more or less drawn-out neck, the phialide ; Phialideæ. (In a fourth division Prophialideæ, the phialide is borne on a differentiated structure which intervenes between the mycelium and the phialide.)

Vuillemin (1911) placed Aleurismeæ at the base of the Conidiosporeæ, but in 1925 classed them in the Arthrosporeæ. Ota and Langeron's family, Closterosporeæ (1923), erected for the reception of *Trichophyton* and its allies, is regarded as unnecessary by Vuillemin, the genera finding their proper place in Arthrosporeæ. These are minor details of classification, perplexing to non-systematists and necessary to note for that reason. They have their explanation in the efforts to construct a reasonable classification.

SPOROTRICHEÆ.

Mycelium septate, usually very slender ; conidia oval or rounded, arising either in clusters at the ends of the mycelial filaments or isolated and irregularly arranged along the whole filaments, finally more or less completely covering them ; conidia sessile or borne on a short sterigma or on a tooth-like persistent process of the mycelial wall.

This family takes its name from the genus *Sporotrichum* Link, but there is some doubt about the proper application of this name. The first isolation of a pathogenic fungus of the group was by Schenk (1896), and E. F. Smith provisionally placed it in *Sporotrichum*. In this he has been followed by practically all other workers. *Sporotrichum* is a genus with very many species mostly incompletely known. The genus is not clearly distinguished from allied genera with similarly badly defined species. The fact that almost two hundred of these reputed species are described in mycological literature gives an idea of the confusion that exists. Fortunately, only about twelve species concern us here, but, unfortunately, it has been thought proper to alter their nomenclature without due consideration of the problems involved.

Vuillemin holds that *Sporotrichum* Link should be restricted to colourless or light-coloured fungi and transferred the eight pathogenic species referred to it to *Rhinocladium* Saccardo and Marchal, incidentally emending the characters of this genus. It is obvious that the pathogenic species cannot be considered apart from the saprophytic ones, and from a nomenclatural point of view we have to consider the emendation of Link's genus made by Fries. To avoid confusion I have retained the name of *Sporotrichum* in a wide sense.

Sporotrichum Auct.

Sporotrichum Link emend. Vuillemin. Mycelium and spores colourless or brightly coloured ; conidium sessile or borne on a fine sterigma.

Rhinocladium Saccardo and Marchal emend. Vuillemin. Mycelium and spores at length fuliginous ; conidium borne on a tooth which persists after the fall of the spore.

Sporotrichum Beurmannii Matruchot & Ramond.

Mycelium creeping, 2 to 3μ in diameter, septate, colourless, abundantly branched and twisted ; spores in masses about 10μ wide at the ends of the somewhat narrower branched filaments, but arising singly, usually in large numbers on each segment of the filament ; while still attached the spore is pyriform, 1 to 2μ by 0.5μ , prolonged into a stalk which is borne on a very fine sterigma ; when detached becoming oval and brown 3 to 5μ by 2 to 4μ .

In culture from pus and in lesions yeast-like forms occur. Those in lesions are very variable and range from less than 1μ to over 18μ . According to Tour they arise from the budding of conidia, and not by the breaking up of the mycelium which is also present. Causes sporotrichosis, gummatous-like swellings in the subcutaneous tissue. Diagnosis may be made by spore-agglutination. The fungus was found three times (out of fifty-seven gatherings from plants) on oak, horsetail and oats by de Beurmann and Gougerot. This would indicate that it is a common saprophyte. It has been stated by Csontos (1927) that an examination of stale eggs in Hungarian markets showed that 20 to 50 per cent. have a species of *Sporotrichum* similar to, if not identical, with *S. Beurmannii*.

Trichosporium Fries.

Mycelium, well-developed, creeping, occasionally branching, fuscous or light coloured ; conidia globose or ovoid, smooth or very slightly rough, in groups at the ends of the mycelial filaments.

Trichosporium Pedrosi (Brumpt) Brumpt.

Mycelium 2.4 to 3.2μ in diameter, septate with segments 17 to 24μ long. Conidia elongate, 4.5 by 1.5μ , borne on aerial hyphæ.

On Sabouraud's medium forms yeast-like cells at the beginning of growth. Causing dermatitis verrucosa in Brazil. At first confused with *Phialophora*. In scrapings from lesions seen as isolated, or grouped, large, deep brown, yeast-like cells, 4.8 to 8μ by 3.6μ , often septate.

*SPOROPHORÆ.**Acremonium* Link.

Mycelium creeping, sparingly branched, bearing simple (or rarely branched) lateral sporophores ending in a single colourless or slightly coloured spore.

Acremonium Potronii Vuillemin.

Mycelium branched, septate, scarcely 1μ in diameter, hyaline, often bunched in an *Isaria*-like manner; conidiophores simple, normally divaricate, rarely branched, flask-shaped but without a basal wall; spore-bearing portions elongate, 15 to 20μ long, swelling about equal in length to the neck, which narrows upwards and has a truncate apex; conidia successively formed and detached, pink, ovoid, shortly apiculate at the base, 4 to 5μ by 2 to 2.2μ . Not seen in lesions. Easily grown on most media. On Sabouraud's medium the culture is downy, at first white, then pinkish, then orange-yellow.

Produces a soft gumma. Pathogenic to guinea-pig.

*PHIALIDÆ.**Aspergillus.*

Vegetative mycelium consisting of septate, branching hyphæ, colourless, brightly coloured or occasionally turning slowly brown in localized submerged areas, or producing brown crusts or sclerotia; conidial apparatus developed as stalks and heads from specialized enlarged thick-walled hyphal cells (foot-cells), producing conidiophores as branches approximately perpendicular to the long axis of the foot-cells; conidiophores continuous or septate, usually enlarging upwards and broadening into elliptical, hemispherical or globose vesicles bearing sterigmata (phialides), either parallel or clustered in terminal groups or radiating from the entire surface; sterigmata either in one series or as a 'primary' series, each bearing a cluster of two to several secondary sterigmata at the apex; conidia vary greatly in colour, size, shape and markings, are successively cut off from the tips of the sterigmata by cross-walls and form unbranched chains arranged into radiate globose heads or packed into columnar masses. Perithecia found only in a few species: cleistocarpic, thin-walled, enclosing subglobose or pyriform asci with four or eight lenticular spores. Sclerotia occur in some species, occasionally in some strains and not in other closely related strains, commonly composed of thick-walled cells apparently filled with stored material.

There seems no good reason for maintaining the genus *Sterigmatocystis*. Cramer proposed it for a black fungus (*S. antacustica*) found in the human ear, in which the primary series of sterigmata branch so that each sterigma bears a tuft of secondary sterigmata. There are groups of species with constantly one series of sterigmata and others with two, but there are also species in which heads of both kinds are found in the same culture and sterigmata of both kinds in the same head.

In text-books of mycology this genus is usually treated under the generic name, *Eurotium* de Bary, a name applied to the cleistocarpic or perfect stage. This is correct from the point of view of nomenclature, but inconvenient when dealing with the very numerous conidial stages. *Diplostephanus* was proposed by Langeron to indicate the perfect stage of *Sterigmatocystis*.

A large number of species of *Aspergillus* are mentioned in connection with human pathology, mainly otomycoses but also mycetomas and bronchomycoses. The identification of these species is usually very unsatisfactory. 'The culturing of micro-organisms by those trained in medicine has commonly been carried out on media devised for pathogenic bacteria. Such media contain animal substances rather than the carbohydrates which are especially favourable for mould growth. A mould may grow on the substances of the animal body as a pathogenic organism or as a secondary factor in a pathological condition, but in such an environment it commonly either does not develop its fruiting structures at all or develops such structures differing in form and colour from the usual saprophytic cultures upon which our records and descriptions have been drawn. In such lesions, conidial apparatus, if present at all, is very much reduced; vegetative hyphæ in some species are very slender and delicate, in others become large, vesiculate or even tend to break up into single cells. However uniformly such structures may be produced, comparative culture under the conditions favourable to the usual morphology of *Aspergillus*, is necessary before we can place such species correctly within the group. Although certain species of *Aspergillus* have been studied and described only as found in pathological materials, hence are unknown in culture, most of the forms isolated from such sources have assumed the structures characteristic of saprophytic species when transferred to culture media in fermentable carbohydrate'.*

Several media are satisfactory for growing *Aspergillus* and similar moulds. The one used by Thom is Dox's modification of Czapek's solution agar.

Penicillium.

Vegetative mycelium abundant, entirely submerged or more or less effused, monopodially branching, septate, commonly showing vegetative anastomoses, colourless or secondarily coloured by products of metabolism which frequently also discolour the substratum, never with hyphal walls, brown or dematiaceous; colonies green, yellow-green, blue-green or grey-green or less commonly colourless or in avellaneous to yellowish, reddish, purplish or other shades; frequently discoloured in age, even with spores brown in mass but not dematiaceous. Conidiophores arise as branches from the vegetative mycelium, frequently perpendicular to the vegetative hyphæ, but not showing differentiated foot-cells as in *Aspergillus*, with walls in some species smooth or undifferentiated, in others

* Thom, C. and Church, M. B., *The Aspergilli*, p. 76, and similarly, Thom, *The Penicillia*, p. 136.

more or less conspicuously pitted or roughened from secondary thickening, not dematiaceous. Conidial apparatus forms a brush (penicillus) ranging from a single terminal verticil of conidia-bearing cells (sterigmata) or a terminal verticil or equal branches or metulæ bearing verticils of sterigmata, to complex branching systems ending in verticils of specialized cells (metulæ) bearing single unbranched chains of conidia each cut off as a cylindrical segment from apical, straight, conidia-bearing tube; conidia cylindrical to oval, elliptical or commonly finally globose, smooth or roughened, colourless or variously coloured, especially in mass, but not dematiaceous when examined with the microscope.

Sclerotium or perithecium formation known only in a few species.

The genus is very rich in species. A few have been reported as pathogenic to man.

The name *Carpentales* Langeron proposed for the perfect stage of *Penicillium* corresponding to *Eurotium*, is, like *Diplostephanus*, an unnecessary introduction.

Scopulariopsis Bainier emend. Thom.

Colonies never green; with aerial hyphæ, partly at least in trailing and anastomosing fascicles; conidiophores very short or wanting, commonly borne along the funiculose hyphæ; conidial apparatus partly *Penicillium*-like, partly in reduced and various aggregations of sterigmata and branches or even single scattered sterigmata; sterigmata either *Penicillium*-like or more or less specialized, tapering gradually from a basal tubular section towards a conidium-producing apex from which successive conidia are cut off by cross-walls. Conidia initially more or less pointed at the apex and truncate at the base, with a more or less thickened basal ring surrounding a basal germinal pore, with walls usually thickened and often variously marked and roughened.

Species of *Scopulariopsis* seem to be definitely related to onychomycosis.

The Ringworm Group.

PATHOLOGY.

BY ARTHUR WHITFIELD.

Introduction.

In dealing with fungous infections of the skin and its appendages, one is at once met by the difficulty that in the older investigations the fungi were classified according to the signs which they produced, and in the newer mycological work they have been classified according to the nature of their growth and fructification when cultivated away from the animal tissues. To follow the second form of classification when alluding to the appearance of parasitic growth would lead to much repetition, since members of apparently different species may produce parasitic effects almost entirely similar, and, on the other hand, members of the same species may produce entirely different parasitic effects. The

method followed will be, therefore, to use the old classification when indicating the type of lesion produced and the microscopic appearances, but in order to conform with recent researches the mycological species and names included in each group will be given.

Using the older classification the members of the Ringworm group dealt with in this section comprise the genera *Microsporum*, *Trichophyton* (including *Endothrix* and *Ectothrix*), *Favus*, *Epidermophyton* and *Endodermophyton*.

History.

Schoenlein (1839) appears to have been the first definitely to associate favus with a hyphomycetous fungus. Gruby (1841) rediscovered the presence of fungus in favus and published an accurate description of it. In 1842 Gruby discovered the fungus of ringworm of the beard of man; in 1843 the fungus now known as *Microsporum* of the scalp and in 1844 that now known as *Trichophyton endothrix*. Malmsten (1848) published work on the subject and introduced the name of *Trichophyton*.

As regards the contagiousness of the disease, Ernst (1820) showed that the skin disease of the cow was transmitted to a girl. Friedberger (1876) described infection of a child from a dog.

According to Sabouraud, Grawitz was probably the first to obtain pure cultures of *Favus* and *Trichophyton*. The next important step was taken by Sabouraud (1894), who devoted many years to observation, culture and classification of the ringworm group. Sabouraud's publications were followed by those of many others, among the chief being Fox and Blaxall, Adamson, Castellani, all working on the lines of Sabouraud's classification. More recently, newer mycological classifications have been suggested by Ota and Langeron and by Grigoraki, and new species of fungi are frequently being found in different countries.

The Microsporum Group.

The lesions produced by this clinical group include infection of the glabrous skin and infection of the scalp hair. One only, *Microsporum equinum* of Sabouraud, has been found in infection of the beard hair, none infect the nails. The commonest group in this country is *M. Audouini*, which may be taken as the type, but *M. felineum* is met with fairly frequently, more so apparently than in France. On the other hand, *M. lanosum (canis)* is much less common in this country.

Lesion of the glabrous skin.

In infection with *M. Audouini* these are only slight and evanescent, consisting of oval or round, scaly macules scattered on the face and neck of the child whose scalp is infected, and sometimes on the arms of the child's attendant. In infection with the *Microsporum* species of animal origin, *M. felineum*, *M. lanosum* and *M. equinum*, also some of the rarer species whose origin is unknown, large rings of scaly papules may be found, and in some cases superficial pustulation. Deep inflammatory

ringworm is not found on the glabrous skin associated with any of the *Microsporum* species. In this country the occurrence of a case of *Microsporum* ringworm of the scalp associated with, or preceded by, well-marked rings upon the glabrous skin should lead at once to the suspicion of feline origin. Microscopic examination of the scales reveals long thin mycelial filaments but no spores.

Lesions on the scalp and affection of the hair.

It is, I think, unanimously agreed that infection of the superficial horny layer precedes that of the hair. After about four days from inoculation there develops a macule with thick glistening scales; this spreads rapidly and may be half an inch in diameter at the end of ten days. Further spread of the original patch may extend to a diameter of two inches. Fresh patches appear on the scalp as a rule, and these are usually found surrounding the original patch, so that by the extension of the old and new patches intersection of circumferences occurs and the original circular arrangement disappears more or less. Within the first ten days, as a rule, the hair is infected and almost every hair becomes broken off about one-eighth of an inch above the scalp level.

In the early stage the fungus is easily found in the surface horny layer, but after two or three weeks, when the hair has become heavily infected, it is difficult to find fungus in the horny layer of the patch though the whole area remains scaly.

Macroscopically the hair is broken, has lost its polish and breaks again easily on attempted epilation. If a whole hair or a large portion of the intrafollicular shaft can be drawn it will be found to be covered with a greyish deposit which turns white if the hair be dipped in chloroform and allowed to dry.

Method of invasion and growth in the hair.

The fungus growing in the horny layer arrives at the follicle mouth and there produces large mycelial ribbons and very large irregular bodies, which are apparently spores. From these elements mycelial filaments grow and rip up some of the cells of the cuticle of the hair and enter the cortex to grow downwards. Other branches grow down in the internal sheath so that the hair in early infection is surrounded with rather large filaments and is also infiltrated with mycelium.

As the hair continues to grow upwards the extrafollicular portion of the shaft carries up its infection, and so shows intrapilar mycelium, but some of this older mycelium seems to wither, as mycelium is usually less abundant in the upper than in the lower portion of the shaft. The growth of the mycelium downwards in the shaft proceeds at a rate greater than that of the hair-shaft upwards, consequently the mycelium reaches nearly the root of the hair; it does not, however, invade the actually growing soft cells of the root of the hair but terminates above these in a fringe of non-septate, twisted and bulbous ended filaments known as 'Adamson's fringe'.

The mycelial filaments branch dichotomously in a downward direction and some of these branches turn outwards, penetrate from within outwards the cuticle of the hair and break up into small spores, which, owing probably to pressure, become angular in outline, forming a mosaic on the outer side of the hair and giving rise to the greyish deposit seen with the naked eye.

Microscopic identification.

The fully infected hair shows therefore :

- (1) A shaft which is infiltrated with septate mycelium, the elements of which are from three to four times as long as they are thick.
- (2) A mosaic of small angular spores on the outside of the shaft, these spores *showing no arrangement in chains*.
- (3) The cuticle of the hair, ripped up and ragged.
- (4) At the lowest portion of the hair non-septate clubbed mycelium, 'Adamson's fringe'.

The disease is limited to children of any age up to sixteen years, after which it dies out spontaneously. Lesions of the glabrous skin in either the child or the adult are shortlived and die out in a few weeks.

Varieties included by clinicians :

Sabouraudites Audouini, synonym *Microsporum Audouini* Gruby (1843).

<i>S. (Closteramma) equinus</i>	„	<i>M. equinum</i> Bodin (1896).
<i>S. flavescens</i>	„	<i>M. flavescens</i> Horta (1911).
<i>S. felineus</i>	„	<i>M. felineum</i> Fox & Blaxall (1896).
<i>S. pubescens</i>	„	<i>M. pubescens</i> Sabouraud (1909).
<i>S. tomentosus</i>	„	<i>M. tomentosum</i> Pelagatti (1909).
		This might be identical with <i>M. Audouini</i> .
<i>S. tardus</i>	„	<i>M. tardum</i> Sabouraud (1909).
<i>S. umbonatus</i>	„	<i>M. umbonatum</i> Sabouraud (1907).
<i>S. xanthodes</i>	„	<i>M. xanthodes</i> Fischer (1918).
<i>S. lanosus</i>	„	<i>M. lanosum</i> Sabouraud (1907).
<i>S. fulvus</i>	„	<i>M. fulvum</i> Uriburu (1907).
<i>S. scorteus</i>	„	<i>M. scorteum</i> Priestley (1914).
		? Identical with <i>S. fulvus</i> .
<i>S. depauperatus</i>	„	<i>M. depauperatum</i> Guéguen (1912).
<i>S. iris</i>	„	<i>M. iris</i> Pasini (1911).
<i>S. velveticus</i>	„	<i>M. velveticum</i> Sabouraud (1907).
<i>S. villosus</i>	„	<i>M. villosum</i> Minne (1907).
<i>Trichophyton minimum</i>	„	<i>M. equinum</i> Le Calvé & Malherte (1898).
<i>Grubyella ferruginea</i>	„	<i>M. ferrugineum</i> Ota (1922).

The Endothrix Group.

The characteristic feature of this group is that in the well-established case of hair infection, fungus is found only within the shaft of the hair,

and the root-sheath shows no fungus. In the early stage of infection mycelium is found also in the root-sheath, but this dies out leaving the hair alone infiltrated with fungus. It follows, therefore, that in a well-established case in which the infection still is spreading a few of the most recently attacked hairs will still show mycelium in the root-sheath outside the hair. There is also a subsidiary group in which the fungus outside the hair is slower to die out, though it does so eventually, and consequently hairs showing fungus on the outside can be found for a longer period. To these species Sabouraud has given the name '*Neo-endothrix*'. The tissues affected by these species are: the glabrous skin, the hair of the scalp and that of the face, the nails and, on rare occasions, the interdigital spaces of the hands and feet.

Lesions of the glabrous skin.

On the glabrous skin the *Endothrix* fungi give rise to bold rings of a bright red colour which vary much in size and may attain a diameter of at least three inches. The ring shows at the edge either red scaling papules, vesicles or superficial pustules. Examination of the scales, roofs of vesicles or pus shows chains of cubical or round elements resembling large spores. Adults are affected and the lesions do not die out spontaneously, at all events in a short time, but as they are treated when seen it is not definitely known how long they might last on the drier portions of the skin. In the rare cases in which varieties of this group infect the interdigital spaces of the palms and soles the duration seems to be unlimited.

A very rare group of cases in one family has been published in which *Endothrix* fungus (*Trichophyton violaceum*) was found to have invaded the skin of the trunk, hands and feet, the hair of the head, the nails, the lymphatic glands in the neck, causing sinuses. The urine contained a few mycelial elements and the fungus was also seen but not cultivated from the vagina in a female member of the family and from scrapings of the tongue in one of the males (Pelévine et Tchernogouboff, 1927).

Lesions of the scalp.

Two varieties of lesion are common, but in rare instances atypical lesions may be produced such as the deep, suppurative ringworm known as 'Kerion'. Of the two types of lesion the first, and in this country the commoner, is a small patch of rather thick white scales. Within this patch the hairs are soon infected and the scaly lesion may then die out, leaving a small area about a third of an inch in diameter within which the hairs are broken off. This breakage occurs much lower down than in *Microsporum* infection and may be level with the surface of the skin; and since in the later stages scaling has disappeared and the patches of broken hairs are small, the disease is often overlooked. Not infrequently there are small indolent pustules surrounding the infected hair follicles and the patches are scattered all over the head.

The second type of lesion is of larger area, covered with very heavy scales and shows no projecting broken hairs. Not every hair within the infected area is attacked, many long and healthy hairs remaining, so that at first sight the hair appears to be merely thinned. On lifting carefully some of the thick scales the softened, brittle, and deformed hairs may be found lying beneath, often twisted into the shape of an 'S' or coiled like a watch-spring.

Lesions of the beard region.

In this position small patches of reddened skin are to be found and close examination shows that the hair included in these patches is broken, and around each broken hair lies a collarette of scale. Slight pustulation is also sometimes present.

Method of invasion and growth in the hair.

As in *Microsporum* the fungus reaches the opening of the hair follicles and the hair by spreading to them from the infected surface horny layer. It then grows down the follicle on the outside of the hair and some mycelium insinuates itself under the cuticle and grows down the hair-shaft, but precisely at what level this invasion of the hair-shaft occurs is not known. After a period which is also unknown, but is certainly shorter in the *Endothrix* species than in the *Neo-endothrix*, the fungus dies away on the outside of the hair, and the infection then lies within the hair-shaft alone. The shaft is then packed with either cubical or rounded elements of the fungus, which appear at first sight to be without definite arrangement, but on closer observation may be seen to be in reality formed into closely packed chains. At the lowest point of the invasion non-septate threads of mycelium may be seen.

Microscopic identification.

The fully infected hair shows :

(1) A shaft infiltrated with cubical or rounded elements, each being as long as it is broad, packed very densely in the hair and arranged in chains which are not very easily seen.

(2) Obvious chains of elements, few in number, on the outside of the hair in the early stages only of the ordinary *Endothrix* and enduring a little longer in the *Neo-endothrix*.

(3) The cuticle of the hair entire or at the most showing damage at one or two points only.

(4) At the lowest point of invasion a fringe of non-septate mycelium. The disease in the scalp is almost limited to children. It dies out a year or two later than the *Microsporum* and is seen rarely in adults.

The disease of the beard region is not apparently limited to any age and it is not known whether it dies out untreated.

Lesions of the nail-plate.

The *Endothrix* and *Neo-endothrix* fungi are the most frequent species found in ringworm of the nail. The posterior nail-fold is thickened and

reddened without pustulation, the nail-bed, especially at the free edge of the nail, is 'hyperkeratotic', and the nail-plate is ribbed longitudinally, deformed, yellowish and friable. On scraping, whitish chalk areas are exposed. The scrapings under the microscope show the fungus in very variable amount; usually the scraping is densely infiltrated with chains of cubical mycelium elements, but occasionally the fungus is so scanty that a prolonged search is necessary.

Species included :

Trichophyton tonsurans Malmsten (1845), synonym *T. crateriforme* Sabouraud.

T. circumvolutum Sabouraud (1909).

T. effractum Sabouraud (1909).

T. exsiccatum Uriburu (1909).

T. fumatum Sabouraud (1909).

T. pilosum Sabouraud (1909).

T. polygonum Uriburu (1909).

T. regulare Sabouraud (1909).

T. Sabouraudi, synonym *T. acuminatum* Blanchard (1895).

T. Soudanense Joyeux (1912).

T. sulfureum Fox (1908).

T. umbilicatum Sabouraud (1909).

T. coccineum Kato (1926).

Ateleothylax Currii, synonym *Trichophyton Currii* Chalmers & Marshall (1912).

Bodinia violacea, synonym *T. violaceum* Bodin (1902).

Trichophyton flavum Bodin (1902), synonym *T. cerebriforme* Sabouraud (1909).

T. plicatile Sabouraud (1909).

These last two form the group named by Sabouraud the *Neo-endothrix*.

The Ectothrix Group.

This name was given to the group by Sabouraud because mycelial filaments could be found in the root-sheath around the hair. Many workers wrongly believed that Sabouraud meant to imply that these fungi did not enter the hair-shaft, and the name was therefore altered later to Endo-ectothrix, but the original name is now more frequently used. All fungi of this group produce marked inflammatory and generally suppurative lesions and in some instances very deep inflammation.

Lesions of the glabrous skin.

The *Ectothrix* fungi in this position give rise to discs of much thickened oedematous inflammation to which the name 'Agminate suppurative folliculitis' was given before the nature of the infection was known. The cushion-like discs are beset with pustules corresponding to the openings of the lanugo hair follicles, and the hair-shafts are loosened. Examination

of loosened hairs reveals the fungus surrounding the hair and expressed drops of pus show masses of mycelium.

Lesions of the scalp and beard region.

In these two regions the clinical appearances are identical, and for this reason they may be described together.

The invasion of the follicles by the fungus sets up an intense inflammation with much serous exudation accompanied by the formation of pus containing ordinary polymorphonuclear leucocytes. Owing to the degree of swelling the lesions have not infrequently been mistaken for those of vaccinia, especially as the host is generally in contact with animals, and have also been erroneously diagnosed as gummatous in origin. The hair of the affected areas is loosened and may be drawn entire with its root-sheath, but it is also rendered brittle so that some broken hairs are usually revealed by a careful search.

This form of ringworm, commonly known on the Continent as 'Trichophytosis profunda' and in this country as 'Kerion', produces some constitutional disturbance with the consequent production of immunity reactions, to which reference will be made below.

The disease usually runs a course which is limited spontaneously in the human being, and it is easily curable, owing to the fact that, as the hair can be drawn entire, one may strip the infected area of all infected hair-shafts, after which simple fomentation with suitable antiseptics completes the cure. Some cases on the Continent appear, however, to offer greater resistance to treatment.

Method of invasion and growth in the hair follicle.

This group of organisms shows so marked a predilection for the hair follicle that the beginning of pustulation is usually the first sign to be seen. The fungus proliferates chiefly in the internal root-sheath and insinuating itself beneath the cells of the cuticle also grows down the hair shaft. Leucocytes stream between the cells of the external root-sheath, accompanied by exudation of serum so that the hair when drawn shows a swollen root-sheath with adhering pus. This swollen root-sheath is packed with cubical mycelial elements which vary in size in the two subspecies which Sabouraud has named *Ectothrix microides* and *megasporos*. In *E. microides* the fungus outside the hair, owing to the comparatively small size of the elements, may be confused with the spores of the *Microsporum*, but this is a mistake which should not be made, since careful examination will show at once that these elements are really arranged in closely set long chains in *E. microides* and not packed into a mosaic without geometrical arrangement as in *Microsporum*.

Once the fungus has arrived within the hair-shaft it would appear that not much further attack occurs from without, as the cuticle edge of the hair is almost entirely intact. Within the hair the mycelium grows in long chains of cubical elements which vary much in abundance in different cases and probably with the different species.

Microscopic identification.

The fully infected hair shows :

(1) A shaft infiltrated to a variable extent with chains of cubical mycelial elements.

(2) A very dense system of straight and wavy or winding chains of similar mycelium on the outside of the hair.

(3) The cuticle of the hair-shaft for far the greater part of its length is undisturbed and sharp in outline. (Important distinction from *Microsporum*.)

(4) At the lowest portion of the intrapillary growth a fringe of non-septate mycelium.

The disease is not entirely limited to children, even on the scalp.

Species included.

The species belonging, under the new classification, to the above clinical group are :

Trichophyton denticulatum Sabouraud (1910). (*T. denticulatum* Sabouraud belongs to his group *microides niveum*.)

T. equinum Gedoelst (1902). (*T. equinum* Matruchot belongs to Sabouraud's group *megaspores*.)

T. erectum Horta. (Belongs to Sabouraud's *microides gypsum* group.)

T. felineum Blanchard (1895). (*T. radians* Sabouraud belongs to his group *microides niveum*.)

T. megnini Blanchard (1895). (*T. rosaceum* Sabouraud belongs to his group *megaspores*.)

T. vinosum Sabouraud (1909). (*T. vinosum* Sabouraud belongs to his group *megaspores*.)

Sabouraudites (Aleurocloster) asteroides. (*T. asteroides* Sabouraud belongs to *microides gypsum*.)

Sabouraudites (Aleurocloster) caninus Matr. & Dassonville (1902). (*T. caninum* Mat. belongs to Sabouraud's group *megaspores*.)

Sabouraudites (Aleurocloster) farinulentus Sabouraud (1910). (*T. farinulentum* Sabouraud belongs to his group *microides gypsum*.)

Sabouraudites (Aleurocloster) granulosus Sabouraud (1908). (*T. granulosum* Sabouraud belongs to his group *microides gypsum*.)

Sabouraudites (Aleurocloster) griseus Vasconcellos (1914). (*T. griseum* Vasconcellos probably belongs to Sabouraud's group *microides*.)

Sabouraudites (Aleurocloster) radiolatus Sabouraud (1910). (*T. radiolatum* Sabouraud belongs to his group *microides gypsum*.)

Sabouraudites (Aleuramma) lacticolor Sabouraud (1910). (*T. lacticolor* Sabouraud belongs to his group *microides gypsum*.)

Sabouraudites (Aleuramma) persicolor Sabouraud (1910). (*T. persicolor* Sabouraud belongs to his group *microides gypsum*.)

Sabouraudites (Aleuramma) Viannai de Mello (1917). (*T. Viannai* de Mello probably belongs to Sabouraud's group *microides*.)

Grubyella alba Sabouraud (1909). (*T. album* Sabouraud belongs to his group *megaspores* with faviform cultures.)

Grubyella discoides Sabouraud (1909). (*T. discoides* Sabouraud belongs to his group *megaspores* with faviform cultures.)

Grubyella ochraceum Sabouraud (1909). (*T. ochraceum* Sabouraud belongs to his group *megaspores* with faviform cultures.)

The Epidermophyton Group.

This name was given to a relatively small group of fungi which attack the horny layer and the nails but do not attack hair.

Lesions are in the form of (1) a marginate eczematoïd dermatitis of the main flexures, the inguinal and the axillary regions, and (2) vesicular dermatitis of the toes and soles, less frequently the fingers and palms, and in both positions infiltrating the nails in long-standing cases. In the cases affecting the extremities the disease is extremely difficult to eradicate, probably on account of the thickness of the horny layer.

Species included.

The species included under this group according to the new classification are :

Epidermophyton cruris Castellani (1905), said to be identical with *Epidermophyton inguinale* Sabouraud (1907). I have doubts about the identity of the fungi described under these two names. Castellani describes his fungus as lemon-yellow and in the cases in which I have succeeded in getting pure cultures this is an accurate description. The surface of the culture is also dry, downy and somewhat powdery and shows no special folds. On the other hand, the cultures of Sabouraud's type which I have received from his laboratory and those I have succeeded in cultivating myself are of very characteristic form showing large radiate folds like the spokes of a wheel and a heaped-up summit with a small central crater, the surface is moist and not powdery, and the colour a dirty greyish buff to brown.

Epidermophyton Perneti Castellani (1907).

Epidermophyton repens Eklund (1883).

Epidermophyton rubrum Castellani (1907) = *Sabouraudites rubra*.

Epidermophyton clypeiforme McCarthy (1925).

Epidermophyton interdigitale Priestly (1917).

Epidermophyton plurizoniforme McCarthy (1925).

Epidermophyton lanoroseum McCarthy (1925).

Epidermophyton gypseum McCarthy (1925).

Epidermophyton niveum McCarthy (1925).

The position of these fungi is not settled under the classification of Langeron and Ota as are most of those already mentioned under the other groups.

The Endodermophyton Group.

This is a small group of tropical fungi, one instance of which originally was found and described accurately by Manson under the name of Tokelau Ringworm. They attack the surface horny layer only and produce curious watered-silk-like patterns.

Species included :

Endodermophyton concentricum Blanchard (1895).

Endodermophyton indicum Castellani (1911).

Endodermophyton Mansoni Castellani (1914).

Endodermophyton tropicale Castellani (1914).

The Favus Group.

The clinical characteristics of this group are as follows : They attack persons of all ages, but are not very actively contagious. They invade the glabrous skin, the hair and nails, and, in one recorded case the mucous membrane of the stomach was affected. They produce on the glabrous skin and scalp small crateriform lesions, so-called scutula, of a bright canary colour and mouse-like odour. They cause scarring and permanent loss of hair without suppuration in long-standing cases.

Lesions of the glabrous skin.

These occur usually in the form of scaly patches with some inflammatory redness surrounding them. On the surface of the area is scattered a variable number of scutula.

Method of growth.

Microscopically *Favus* differs in its method of growth from all the other fungi. The mycelial chains, instead of growing out in a horizontal plane, arrange themselves nearly vertically, like a forest of saplings, at the superficial aspect of which the fructification gives rise to masses of spores.

Method of invasion and microscopical appearance in the hair.

The hair is attacked by the growth from the scutulum growing around and into the hair follicles and the hair. It then produces outside the hair large cubical and polygonal elements and long mycelial threads of very variable thickness. Within the hair long mycelial chains with elements usually of much greater length than their thickness are seen following a waving course in the hair-shaft and branching trichotomously and possibly tetrachotomously downwards.

Microscopic examination of the hair for identification shows therefore :

- (1) A hair usually not broken but epilated in its entirety.
- (2) Very irregular masses of protoplasm and mycelial chains in the internal root-sheaths.
- (3) The cuticle of the hair intact.
- (4) The shaft of the hair infiltrated with long strands of sinuous mycelium branching trichotomously downwards.

Invasion of the nails.

Invasion of the nails is less common in favus than in trichophytosis. The disease shows itself as a yellow blotch in the nail-plate in the early stages. Later, the plate is disintegrated. Mycelium is found abundantly in scrapings of the diseased nail substance.

Species included by clinicians.

Sabouraudites (Aleurocloster) gallinæ Méguin (1881), synonym *Achorion gallinæ* Sabouraud (1910).

Sabouraudites (Aleurocloster) gypseus Bodin (1907), synonym *Achorion gypseum* Bodin (1907).

Sabouraudites (Aleurocloster) violaceus Bloch (1911), synonym *Achorion violaceum* Bloch (1911).

Sabouraudites (Closteramma) Quinckeanus Zopf (1890), synonym *Achorion Quinckeanum* Zopf (1890).

Bodinia annulosa Cazalbon (1914), synonym *Achorion annulosum* Cazalbon (1914).

Grubyella Schoenleinii Lebert (1845), synonym *Achorion Schoenleinii* Lebert (1845).

IMMUNITY, SENSITIZATION AND SYSTEMATIC INFECTIONS.

BY ARTHUR WHITFIELD.

The chief work on immunity was done by B. Bloch, but other investigators have followed. Some of the conclusions arrived at by different workers have been contradictory to one another, but the following appears to be on the whole well established.

No immunity is produced by the more superficial infections such as are usually caused by the clinical class of *Microsporum*. According to Bloch, immunity is produced by the deep pyogenic infections mostly due to the clinical group of *Ectothrix-Trichophyton*. Bruhns and Alexander (1910), on the other hand, found that *Trichophyton gypseum* did not confer immunity on man, but that reinfection could occur after six months. It is noteworthy that Bloch's cases were deep infections, whereas Bruhn's and Alexander's were 'moderately superficial', an unusual state of affairs with *T. gypseum*. Bloch manufactured antigens from various species (of *Trichophyton* and *Favus*), and found that their action was not specific, infections by any species giving reactions with any 'trichophytin'. He also found that human skin transplanted from a sensitized to a normal individual retained its sensitization in the new position and would react there. Bloch came to the following conclusions:

(1) There are *Trichophyton* fungi which give rise to the typical disease on inoculation.

(2) All inoculated animals are subsequently immune over their whole cutaneous surface.

(3) This immunity is not specific among the species; widely separated cultures will give rise to it.

(4) In man deep Trichophytosis probably causes immunity.

(5) In man all deep Trichophytosis gives rise to allergy, producing papules on inoculation of the filtrates of old cultures, and this allergy appears to last a long time.

(6) Allergy, like immunity, is not specific, any member of the *Trichophyton* group or *Achorion* group will be found active.

(7) Immunity and sensitization show that *Achorion Quinckeanum* is more closely related to the *Trichophyton* group than is *Achorion Schcenleinii*.

Citron found that the production of precipitins could be excited, but not agglutinins.

Systemic infection has been seen in man chiefly with infections of the deep pyogenic fungi. Finnerud described it in an epidemic apparently of *Microsporum Audouini* in Vienna, but although his cultures were characteristic of this fungus the lesions on the scalp were far more inflammatory than those usually seen with it in this country. The systemic infection is shortlived and does not appear to affect the general health materially. The sign of it is the appearance of a widespread eruption of small papules, sometimes arranged in discs and small circles and subsequently scaling. To this eruption the name 'trichophytide' has been given by analogy with systemic eruptions of syphilis and tuberculosis, called 'syphilides' and 'tuberculides'. One or two observers have claimed to have obtained cultures of the fungus from the blood in patients the subject of 'trichophytide'.

A. W.

CULTIVATION.

Although it is possible with experience to give a shrewd guess at the identity of a fungus causing a lesion on microscopical examination of a diseased hair, it is necessary to cultivate the fungus for a proper determination. Cultivation was first attempted by Gruby (1842), who said that he had been able to grow the fungus of favus on wood. Remak (1845) placed the spores of *Achorion* on a series of diverse media and obtained evident germination on apple after forty-eight hours. His drawings show that he was apparently dealing with *Achorion*, but his cultures became covered with *Penicillium* after six days. This was at the beginning of the classical period of the controversies about the pleomorphism of fungi. The early stages of this controversy had centred about spontaneous generation, but with increased knowledge of fungal morphology consequent on the use of better microscopes and with attempts at cultivation, the controversy entered on another stage which ended only when the methods chiefly associated with the name of Pasteur were rigidly applied. A history of the subject from 1840 to 1870 would be more or less that of the mycology of the period, and it is probably because of the wild extravagances of writers such as Hallier that medical mycology was for some time regarded generally with disfavour. These 'observations', as de Bary says, belong to the scientific 'chronique scandaleuse' and need no further mention beyond stating that Hallier,

after regarding *Penicillium* and *Achorion* as the same fungus, later held that the second arose from the smut *Ustilago Carbo*. Köbner (1864) sought for the solution of the problem by showing that *Trichophyton* when inoculated produced the disease, whereas *Penicillium* had no effect.

Apparently Grawitz (1886) first obtained pure cultures of *Trichophyton* and *Achorion* by sowing them on gelatin, agar and blood-serum and using bacteriological methods. A few days later Duclaux confirmed these results in a paper giving an account of the researches he had undertaken to disprove Grawitz's previous statements (1878) that *Achorion*, *Trichophyton* and the organism of pityriasis were diverse forms of *Oidium lactis*. The methods of pure culture now began to be applied generally and soon the idea held by certain clinicians that there were many different species of fungi capable of causing lesions was abundantly proved.

Many different culture media were described by investigators until there was a general adoption of Sabouraud's medium. Nowadays it is frequently overlooked that these fungi will grow on most of the common laboratory media. If a ringworm fungus is sown on a series of media, including those most used by mycologists, startling differences in the gross appearances are revealed; also temperature and light occasionally have a considerable effect. As Sabouraud has pointed out, however, the appearance is definite on a given medium, being related to its chemical composition and changing with it. He proposed the formula associated with his name* because the medium gave luxuriant and rapid growth, together with clearly marked differentiation. It is to be remarked, however, that it does not necessarily give the optimum growth for every species of dermatophyte. This is important, for the different species have been diagnosed mainly on cultural and morphological differences. Judging from the literature, it is necessary to stress that, though Sabouraud's medium is invaluable, it is unreasonable to assume that the whole of the morphology of a fungus must of necessity be revealed by it. The medium should always be used as a routine, but for descriptive work, as apart from diagnosis, a series of media should be employed. Thus Sabouraud has recently stated that a more satisfactory medium is 1 per cent. peptone, 8 per cent. honey, 100 parts of water and 2 per cent. agar, for he found that on it *Achorion Schoenleinii* formed aleuria and rudimentary spindles with and without septa.

CLASSIFICATION.

The classification of the fungi of this group has received considerable attention during the last few years. Following the investigations of

* This was originally arrived at by empirical modification of the figures given by analysis of beer-wort. The first 'formule générale' (1894) was: sugar, 3.8 gm.; peptone, 0.5 to 0.8 gm.; distilled water, 100 gm.; agar-agar, 1.4 gm. The formula was afterwards given more precisely as follows, as it was found that different brands of sugar and peptone gave different cultural characters: crude maltose (Chanut), 4 gm.; granulated peptone (Chassaing), 1 gm.; distilled water, 100 gm.; agar-agar, 1.8 gm.

(1) Humid and glabrous; characterized especially by chains of arthrospores. *Achorion Schoenleinii*, *Trichophyton violaceum*, *Trichophyton* of the faviform type, *Microsporum ferrugineum*, &c.

(3) Downy at first but later with a powdery surface : showing most complete development of spore-forms, aleuriospores, pluriseptate fusiform spores, &c. *Trichophyton gypseum* type, *T. radians*, *T. denticulatum*, *T. lacticolor*, &c., and animal microsporums.

Thus, the microscopic characters can be presumed solely on the external appearance of a culture when grown on Sabouraud's medium. Ota and Langeron (1923) have proposed a classification based on the microscopic characters of the fungi without reference to the lesions. The characters they rely on are indicated in the following key :

- | | |
|--|------------------------|
| (1) Aleuriospores numerous, well developed, isolated or in clusters. | 2 |
| Aleuriospores rare and imperfect, isolated, rarely in clusters. | 3 |
| (2) No fusiform spores or accessory organs. | <i>Trichophyton</i> |
| Fusiform spores and other accessory structures (nodular, pectinate, spiral, &c.) | <i>Sabouraudites</i> |
| (3) Numerous and well characterized fusiform spores. . . | <i>Epidermophyton</i> |
| No fusiform spores. | 4 |
| (4) Arthrospores accompanied by accessory structures (clavate, terminal chlamydospores, &c.) | <i>Grubiyella</i> |
| Arthrospores without accessory structures. | 5 |
| (5) Arthrospores in chains. | <i>Bodinia</i> |
| Arthrospores ill defined. | <i>Endodermophyton</i> |

(1) Aleuriospores and fusiform spores.....	2
Aleuriospores and nodular structures but no fusiform spores....	
	<i>Aleuramma</i>
(2) Nodular structures.....	<i>Closteramma</i>
No nodular structures.....	<i>Aleurocloster</i>

There can be no doubt that the true way to ascertain the proper mycological data by which to arrange the species is to study the microscopic characters shown in culture. The classification proposed by Ota and Langeron, however, can be regarded only as a preliminary attempt, for only one culture medium, Sabouraud's, has been used in their studies. As a consequence the just criticism may be made that the new genera proposed are too artificial, for they are dependent for their characterization on this medium: a single medium is frequently extremely useful for distinguishing between species, but it is illogical to restrict a classification to the characters which are the resultant of it.

In my opinion too much has been made of the artificiality of the characters to be derived from lesions: the fault does not lie here so much as in the prevalent notion that a description of the lesion alone without precise characterization of the causal fungus is sufficient. Many fungi causing plant diseases are distinguishable at sight and the fact that certain genera always cause the same sort of disease may be a 'character' useful in classification. The mode of growth in a hair or on the skin when it is for the most part constant, i.e. the most important fact for the medical man, is therefore not one which should be neglected by the mycologist. It does not appear that one is justified in regarding growth on a given artificial medium as of more scientific value than one based on 'natural' growth. Grigoraki* (1924) proposed another classification which in its most recent form (1929) has undergone considerable modification. He now diagnoses *Microsporum*, *Trichophyton* and *Achorion* as follows:

Microsporum. Cultures originally powdery and with fusiform spores.

Subgenus *Closterosporium*. With numerous fusiform spores (*M. lanosum*).

Closteraleuriosporia. Not markedly powdery (rapidly becoming downy) with few fusiform spores and aleuriospores of variable size (*M. Audouini*).

Trichophyton. Cultures downy with chlamydospores and aleuriospores (*T. crateriforme*).

Subgenus *Chlamydoaleuriosporia*. With large chlamydospores and aleuriospores (*T. crateriforme*).

Aleuriosporia. With aleuriospores (*T. acuminata*).

Achorion. Cultures glabrous and humid reproducing by arthrospores (*A. Schoenleinii*).

Subgenus *Grubyella*. Arthrospores multinucleate with much glycogen (*A. Schoenleinii*).

Bodinia. Arthrospores with few nuclei (*Trichophyton violaceum*).

As it stands this grouping is little different from that which can be traced in Sabouraud's accounts, but the confusion introduced in nomenclature by Grigoraki's writings necessitates a reconsideration of the basis of

* Written Grigorakis in his later works.

classification and that the application of the older generic names should be made more precise. Vuillemin (1925), impatient of the tinkering with these and in the attempt to preserve their medical significance, has gone so far as to suggest that certain generic names well known in general mycology would serve the purpose of the reformers.

A schema in which Sabouraud has focused the two aspects of classification is reproduced.

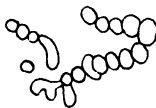
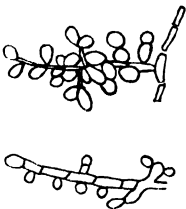
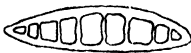
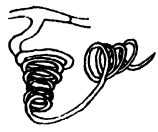
PLEOMORPHISM.

A very common phenomenon in cultures of ringworm fungi is that which was called pleomorphism by Sabouraud. Many of these fungi when grown on media containing carbohydrates, suddenly change their mode of growth. The commonest type on Sabouraud's proof medium is a white downy growth (duvet) on the surface of the mature colony. These tufts may arise anywhere on the surface and usually soon cover it owing to their rapid growth, and so mask the normal colony. When Sabouraud first encountered the phenomenon (1892-4) he was under the impression that he was dealing with two fungi which lived together as commensals in the lesion, one of them being pathogenic, and that they separated out when grown in culture. Bodin (1894, 1896), working with *Microsporum equinum*, realized that this view was incorrect and he and Sabouraud (1896) gave the proper interpretation.

It is unfortunate that the term 'pleomorphism' should have been used by Sabouraud and that he should have been followed by all writers on the subject. As previously stated, Tulasne coined the term to express the occurrence of two different stages in a life-history. The present phenomenon apparently has little in common with pleomorphism as usually understood, but seems to be a form of saltation. There is a rapidly accumulating mass of data on saltation in fungi grown in culture, but unfortunately most of it merely records the fact that a visible change has occurred in the manner of growth or colour of a colony and the media on which the change may be induced. We are ignorant what these changes really are and what they signify. An intensive study of cultures of ringworm fungi from a purely mycological point of view would be most valuable both to mycologists and to clinicians.

It is to be noted that in addition to the downy growth commonly described, sectoring of the more usual type also occurs. Sabouraud, for example, records that in *Trichophyton violaceum* a sector of a culture instead of being humid and glabrous became dry and downy, and well-marked sectoring has occurred in several of my cultures. Further, it is to be noted that in *Microsporum lanosum* Sabouraud described three different saltant forms, a downy, white, compact growth, an immersed growth with a smooth, glabrous, moist, brownish surface, and a velvety shaggy growth which is the most stable and the most frequent: the three forms can be converted one into another, but not into the original form.

CLASSIFICATIONS PROPOSED BY MYCOLOGISTS FOR THE RINGWORM PARASITES.
(Read vertically.)

<i>Grubyella</i> Ota & Langeron, 1923. <i>Bodinia</i> Ota & Langeron, 1923. <i>Arthrosporia</i> Grigoraki, 1925. <i>Mycoderma</i> Persoon, 1822 (Vuillemin, 1925).	<i>Trichophyton</i> sensu stricto, Ota & Langeron, 1923. <i>Aleurosporia</i> Grigoraki, 1925. <i>Aleurisma</i> Link, 1816 (Vuillemin, 1925).	<i>Sabouraudites</i> Ota & Langeron, 1923. <i>Closterosporia</i> Grigoraki, 1925. <i>Fusoma</i> Corda, 1837 (Vuillemin, 1925).	<i>Sabouraudites</i> Ota & Langeron, 1923 <i>pro parte</i> . <i>Spiralia</i> Grigoraki, 1925.			
						
Arthrospores	Aleuriospores.		Spindles.		Spirals.	
	Single.	Cluster.	Small.	Medium.		Large.
	+		+	+		+
	+	+				
	+	+	+			
	+					
+	+		+	+	+	
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+	+					
+						

Biltris (1929) has paid special attention to the effects of the acidity of the medium on growth in culture. He states that both the microscopic and macroscopic appearance of a ringworm fungus can be altered by merely changing the acidity of the medium. Working with a fungus isolated from a mouse, he found that out of seven inoculations on glucose-agar, five gave appearances similar to the original culture (obtained from an arm), and two gave very different cultures which appeared to be distinct species. One of these was stable, the other produced sectoring as well as a downy growth. Investigating the one which was ordinarily stable he found that by diverse treatment he was able to obtain a series of forms. The fungus becomes modified on a glucose medium and the changes are classified as follows :

(1) Latent mutations which are revealed by culturing on 3 per cent. peptone.

(2) Obvious mutations which arise at the periphery of the cultures.

(3) Mutations which arise from the reciprocal influence of cultures of the same fungus inoculated close together.

Sabouraud found that when increased amounts of peptone were added to his proof medium pleomorphic changes became fewer and less constant. This led him to propose a conserving medium which was constituted as follows : granulated peptone (Chassaing), 3 to 5 gm. ; distilled water, 100 gm. ; agar-agar, 1.8 gm. In the hands of Sabouraud and his co-workers this medium has been efficacious in retaining the original cultural characters of these fungi. Several times recently he has repeated his contention that this medium preserves the fungi indefinitely, and that he has numerous cultures which show the original spore-forms after many years. Moreover, in considering this subject, it should be noted that a large number of the cultures of ringworm fungi in different laboratories originally came from Sabouraud. On the other hand, cultures in the Centraalbureau voor Schimmelcultures kept on Sabouraud's conserving medium are said to have all become downy.* Grigoraki holds that all species in culture, no matter what the medium, immediately show signs of degradation, cytologically, morphologically and macroscopically. Biltris states that the appearance of changes does not strictly depend on the absence of sugar but on the relation between the amount of carbohydrates and that of albuminoid matter ; the more the second is raised in respect to the first the more stable the fungus appears. The importance of the carbon-nitrogen relation has been much stressed by workers on other fungi. Biltris points out that the conserving medium is reliable only when prepared strictly according to Sabouraud's formula, a conclusion he was forced to through working with peptone derived from different sources.

* 'Les cultures de champignons parasites de l'homme, font le duvet pléomorphe même quand on les cultive sur la peptone Chassaing à 4% d'après les recherches du Prof. Sabouraud. Nos espèces ont été cultivées pendant plus de 15 ans ; les premières années on retient les spores, mais après ils disparaissent'. Letter from Prof. J. Westerdijk, quoted by Grigoraki (1929).

It has frequently been noted that the acidity of a medium changes with the growth of a fungus. With the special form with which Biltris worked the proof medium, originally acid, became alkaline after the first three or four weeks, later acid and then again alkaline, the change being variable in rapidity, depending on the thickness of the medium, an important point in culture work, though frequently overlooked.

The work of Biltris opens up a further line of investigation in determining the factors which bring about the degradation of these fungi. It is unlikely that mere acidity as such is wholly responsible, or that the small changes in chemical constitution brought about by the additions of acids or alkalis would directly occasion such fundamental changes. The matter is not to be regarded as mainly of taxonomic interest, for it remains to be ascertained whether similar changes occur in nature. Having grown a large number of these fungi, I am convinced that changes can be induced in them in various ways. The only difference from common saprophytic fungi so far as I can see is the fact that the change so frequently is to a downy sterile form. Sometimes, as is well known, this change is gradual. The most interesting fact is that the downy sterile form apparently remains so when subjected to ordinary cultural devices, when inoculated into a host produces a similar lesion to that produced by the normal fungus from which it was derived, and when recultured from the lesion is still in its sterile condition.

About sixty species of ringworm fungi have been described. The question arises whether these are species in the generally accepted sense of the term or whether some of them are saltants which have arisen naturally under special conditions. The fact that certain species give normally a downy growth on Sabouraud's medium (cf. p. 64) suggests that they are naturally occurring saltants. Weidman (1926) further has stated that by placing a non-pathogenic yeast on a *Trichophyton* lesion of the toes, a change from a downy type of growth to the powdery type was obtained in cultures.

The problem would appear to be capable of solution by methods of culture and inoculation, for, if in growing any of the different fungi a sector with the macroscopic and microscopic characters of another 'species' arises and this saltant produces lesions similar to those of its morphological equivalents, the probability is that the one arose as a saltant of the other. Until some such investigation has been carried out in detail the question cannot be profitably discussed.

This leads to the possibility of the saprophytic origin of the group. Most writers on the subject have been convinced that these fungi are only secondarily parasitic and that they probably are still to be found growing independently as saprophytes. The ease with which they grow in culture together with the fact that they can be cultivated on feathers, leather, dung and other organic matter, even soil and wood, lends support to this view. Apparently, however, they have never been found occurring naturally on such substrata, and this, at first sight, is somewhat surprising,

for many mycologists have studied coprophilous and similar fungi. However, except for certain very common fungi, coprophilous species are very sporadic and few who would recognize ringworm fungi have been engaged in general mycological investigations. The matter is bound up with the possibility of there being a perfect stage of these fungi. So far only one presumed perfect stage has been found in ordinary cultures, that of *Trichophyton Currii* Chalmers & Marshall 1914 (*Ateothylix* Ota & Langeron, *Matruchotiella* Grigoraki). It is impossible to be certain, however, either that the oval bodies described were perithecia or that they really belonged to *Trichophyton*. Matruchot and Dassonville (1899-1901) brought forward a certain amount of evidence that *Trichophyton* and its allies are the conidial stages of Gymnoascaceæ, a group of primitive Ascomycetes in which the wall of the fruit-body is composed of interwoven protecting hyphæ. Sometimes these are toothed and bear some resemblance to the pectinate hyphæ found in many Dermatophytes. First, homologizing the conidial stages of certain Gymnoascaceæ with *Trichophyton*, they found their greatest support in *Ctenomyces serratus* (*Arthroderma Curreyi*) which occurs on old feathers and amongst decaying leaves. When grown in culture, *Ctenomyces* is very much like a ringworm fungus, and when inoculated into animals is said to produce lesions of the same nature and the same development as ringworm. Later, they isolated a fungus from a ringworm of a dog which gave fruit-bodies in culture; the fungus, *Eidamella spinosa*, is closely related to *Gymnoascus*, and, indeed, has been referred to that genus. Objections have been raised (e.g. by Sabouraud) that the production of lesions by *Ctenomyces* has not been sufficiently substantiated, and that there is some doubt whether *Eidamella spinosa* was the cause of the lesion from which it was isolated. So the matter stood until 1926 when Nannizzi published some interesting observations. Working with a number of Gymnoascaceæ, *Ctenomyces serratus*, *Arachniotus candida*, *A. aureus*, *Gymnoascus Reesii*, *Myxotrichum chartarum* and *M. uncinatum*, he showed that their conidial forms, which previously had received little attention, have great similarity to ringworm fungi. Further, by growing various species of *Trichophyton* on feathers, leather and suchlike in the dark, *T. radiolatum*, *T. asteroides*, *T. denticulatum*, *T. felineum* and *T. equinum* produced perithecium-like bodies, though none of these contained asci. Later, however (1927), he recorded that *Achorion gypseum* on such media at laboratory temperature gave rise to a perfect fruiting stage which he named *Gymnoascus gypseum*.

If these observations are confirmed, observations which are of great taxonomic interest, we shall have fairly convincing proof of the saprophytic origin of ringworm fungi, and, moreover, a clue to their mode of attack, for the majority of Gymnoascaceæ are more or less coprophilous.

That they require confirmation is shown by the results obtained by other workers. Wilencyk (1927-9) has recorded the formation of isolated asci (without an enclosing fruit-wall) in several species of *Trichophyton* and in *Achorion Schoenleinii*. The original method of

obtaining them was by repeated subculturing (eight to ten generations) on Sabouraud's medium, the cultures first being kept moist, but after ten days to a fortnight being allowed to become dry. In his later experiments he found that ascus-formation could be expedited in *Trichophyton granulosum* and *T. equinum* by detaching fragments of three to four days' old cultures in sugar-bouillon and allowing them to dry above the medium at a temperature of 24 or 37° C.: in *Achorion Schoenleinii* slightly older cultures giving evidence of saltation should be taken. Wilenczyk's figures are not satisfactory, and it is impossible to say whether the structures he describes as asci are really such.

Biltris also records isolated asci in cultures of the fungus with which he principally worked. Here, however, the asci appear before the conidial stages when the acidity of the medium is about pH 4·8. Biltris never saw them in hanging-drop cultures. Good photographs are given of the structures. I have tried repeatedly to induce ascus-formation by the methods described, but, though in many fungi I have seen abundance of what I take to be the structures, I have not succeeded yet in finding any with internal spores.

If either of these observers is right in his interpretations of isolated asci with no definite fruit-body we should have to place these fungi in the group which includes *Endomyces* and *Eremascus**. Here again we have to await further research. From a theoretical standpoint it is conceivable that some ringworm fungi might be conidial stages of Gymnoascaceæ and others of Endomycetaceæ, for these are both primitive groups and appear to be fairly closely allied. The possibility that the different fruit-bodies are dependent on the nature of the medium is not one which can be put forward seriously for there is very little resemblance in the type of asci recorded.

PHYSIOLOGY.

The earliest work on the physiology of ringworm fungi is that of Verujsky (1887), who made a comparative study of *Trichophyton* and *Achorion*, and established certain facts which are now the commonplace of laboratory knowledge. Later workers detailed the general growth conditions for the common species.

Roberts (1899) endeavoured to classify these fungi by their proteolytic power. Hairs of man and animals placed in cultures of *Trichophyton* and *Achorion* were attacked to varying degrees. Some species destroy the cuticle and cortex simultaneously, while others first attack the cortex, and later might invade the cuticle or not. A proteolytic enzyme was found to be still active in cultures six years old.

Macfadyen (1895) distinguished in *Trichophyton tonsurans* an enzyme which liquefies gelatin, but could not find either diastase or invertase. The fungus grew on keratin obtained from quills and rendered it more soluble in strong alkali, but a definite enzyme acting on keratin was not demonstrated.

* Biltris, working with a clavis, places them in Ascocorticaceæ—an untenable view.

Bodin (1899-1907) investigated the enzyme reactions of various species. In his last researches on *Achorion gypseum* he records four proteolytic enzymes: trypsin and gelatinase (more abundant in the saltant form), rennin and casease (more abundant in the normal form).

Tate (1929¹ & ²) has investigated the enzymes of *Trichophyton radiolatum*, *T. tonsurans*, *Microsporum lanosum*, *M. Audouini* and *Achorion Schoenleinii*. Normal and saltant forms of *T. radiolatum* were studied comparatively. Peroxidase, catalase, lipase and an indophenol oxidase were present in all the fungi, the catalase being in varying amount. The proteolytic enzyme found in all very much resembles trypsin, for it acts in an alkaline medium, gradually dissolving the clot which it produces in calcified milk, and hydrolyses intact proteins with the production of free amino-acids. It is peculiar in that it does not hydrolyse coagulated egg-albumin in either acid or alkaline media. According to Tate it corresponds to the four enzymes distinguished by Bodin. Proteolytic activity varies considerably, being by far the greatest in *T. radiolatum*. It is noteworthy that no keratolytic ferment was found. Urease occurs in all species except *T. tonsurans*. Maltase and invertase are present in varying degrees, whereas amygdalase is present in all except the normal form of *T. radiolatum*. The general carbohydrase and proteolytic activities of the different species appear to vary inversely to each other, those rich in proteolytic enzymes being poor in carbohydrase and conversely. This is of interest, for *T. radiolatum*, which has much the greatest proteolytic power and is very poor in carbohydrase, grows equally well on peptone and sugar media, but usually shows saltations when there is a high percentage of carbohydrate. The presence of carbohydrates thus greatly influences growth, though judging from the enzymic activities the utilization of carbohydrates is very small compared with that of proteins.

Comparison between the normal and saltant forms of *T. radiolatum* shows that they behave similarly as regards respiratory enzymes, maltase and diastase, but show marked differences in the proteolytic enzyme, for the normal form is considerably the more active, and in invertase and amygdalase, which are very weak in the normal and comparatively strong in the saltant.

From the summary given of the different lines of research it is obvious that with co-ordination they should lead to a satisfactory knowledge of this difficult group.

REFERENCES.

- ADAMSON, H. G., 1895, *Brit. J. Derm.*, **7**, 201.
 ANDERSON, H. W., 1927, *J. Infect. Dis.*, **21**, 341.
 ARNAUD, G., 1918, *Les Astérinées*, Thèse de Paris.
 ASHWORTH, J. H., 1923, *Trans. Roy. Soc. Edinb.*, **53**, 301.
 BARTHELAT, G. J., 1903, *Les mucorinées pathogènes et les mucormycoses chez les animaux et chez l'homme*, Thèse de Paris.
 BENNETT, J. H., 1842, *Edinb. Med. Surg. J.*
 DE BEURMANN & GOUGEROT, 1907, *Bull. Soc. méd. Hôp.*, **24**, 302, 309.
 DE BEURMANN, L. & GOUGEROT, H., 1912¹, *Les sporotrichoses*, *Bull. Soc. franç. Derm. Syph.*, **23**, 222; 1912², *Les nouvelles mycoses*, Paris.

- BILTRIS, R., 1929, *Ann. Inst. Pasteur*, **43**, 281.
- BLAKESLEE, A. F., 1904, *Proc. Amer. Acad. Arts Sci.*, **40**, 205.
- BLOCH, B., 1908, *Arch. Derm. Syph.*, Wien, **93**, 157.
- BODIN, E., 1894, *Ann. Derm. Syph.*, Paris, 3 S., **5**, 1220; 1896, *Les teignes tendantes du cheval et leurs inoculations humaines*, Thèse de Paris; 1899, *Arch. Parasit.*, Paris, **2**, 362; 1902, *Les champignons parasites de l'homme*, Paris; 1904, *Les champignons parasites de l'homme*, Masson et Cie, Paris.
- BODIN, E. & LENORMAND, C., 1901, *Ann. Inst. Pasteur*, **15**, 279.
- BRUHNS, C. & ALEXANDER, A., 1910, *Derm. Z.*, **17**, 695.
- BRUMPT, E., 1905, *Arch. parasit.*, Paris, **10**, 489; 1929, *Précis de Parasitologie*, 4th edn., Masson et Cie, Paris.
- CASTELLANI, A., 1927-8, *Fungi and Fungous Diseases*, Adolph Gehromann Lecture, New Orleans, reprinted from *Arch. Derm. Syph.*, N.Y.; 1928, *Proc. R. Soc. Med.*, **21**, 447 (Sect. Trop. Dis. Parasit., p. 1).
- CASTELLANI, A. & CHALMERS, A. J., *Manual of Tropical Medicine*, Baillière, Tindall & Cox, London.
- CHALMERS, A. J. & ARCHIBALD, R. G., 1916, *Ann. Trop. Med. Parasit.*, **10**, 169.
- CHALMERS, A. J. & MARSHALL, A., 1914, *J. Trop. Med.*, **17**, 257.
- CITRON, J., 1905, *Z. Hyg. InfektKr*, **49**, 120.
- CSONTOS, J., 1927, *Közl. Osszehas. élét.-es kortan Köréböl.*, **20**, 160, cited from *Bull. Inst. Pasteur*, **26**, 457.
- DA FONSECA, O., 1928, *Bol. Inst. Clin. Quirurg.*, **4**, 469.
- DA FONSECA, O. & DE ARÉA LEÃO, A. E., 1928, *Suppl. Mem. Inst. Oswaldo Cruz*, **4**, 124.
- DESENNE, E., 1878, *C.R. Acad. Sci.*, Paris, **87**, 34.
- DUCLAUX, E., 1886, *C.R. Soc. Biol.*, Paris, *Teignes et teigneux*, Thèse de Paris.
- ERNST, 1820, *Arch. Thierhkl Ges. Schweiz. Theraerzte*, cited from Sabouraud, R., 1910.
- FINNERUD, C. W., 1925, *Brit. J. Derm.*, **37**, 63.
- FOX, J. C. & BLAXALL, F. R., 1896, *Brit. J. Derm.*, **8**, 241.
- FRIEDBERGER, 1876, *Arch. wiss. prakt. Tierhkl*, cited from Sabouraud, R., 1910.
- GEDOELST, L., 1902, *Les champignons parasites de l'homme et des animaux domestiques*, Brussels.
- GOUGEROT & CARAVEN, 1909, *Rev. Chir.*, Paris, **40**, 896.
- GRAWITZ, P., 1878, *Bot. Ztg.*, **36**, 410; 1886, *Sem. méd.*, Paris, 13.
- GRIGORAKI(S), L., 1924, *C.R. Acad. Sci.*, Paris, **179**, 1423; 1925, *Ann. Sci. nat. Bot.*, Sér. 10, **7**, 165; 1929¹, *Ann. Parasit.*, **7**, 83; 1929², *Ann. Derm. Syph.*, Paris, **10**, 18.
- GRUBV, M., 1841, *C.R. Acad. Sci.*, Paris, **13**, 72; 1842¹, *ibid.*, **14**, 634; 1842², *ibid.*, **15**, 512; 1844, *ibid.*, **18**, 583.
- GUÉGUEN, F., 1904, *Les champignons parasites de l'homme et des animaux*, Paris.
- GUILLIERMOND, A., 1912, *Les Levures*, Paris.
- HANSEN, E. C., 1889, *Ann. Micrographie*, **2**, 214.
- HORTA, P., 1911, *Mem. Inst. Oswaldo Cruz*, **3**, 86.
- KOBNER, H., 1864, *Virchows Arch.*, **29**, 226.
- LANDRIEU, M., 1912, *Les mycoses oculaires*, Thèse de Paris.
- LANGERON, M., 1922, *Nouveau traité de médecine*, Roger, Vidal et Feissier, **4**, 495; 1928, *Ann. Parasit.*, **6**, 385; 1929¹, *ibid.*, **7**, 145; 1929², *ibid.*, **7**, 309.
- LENDNER, A., 1908, *Les Mucorinées de la Suisse*, Berne.
- LUCET, A. & COSTANTIN, A., 1900, *Rev. gén. Bot.*, **12**, 81.
- MACFAYDEN, A., 1894-5, *J. Path. Bact.*, **3**, 177.
- MACNEAL, W. S. & TAYLOR, R. M., 1914, *J. Med. Res.*, **30**, 261.
- MAGALHÃES, O. DE, 1929, Contribution to the knowledge of lesions caused by the *Oidium brasiliensis* (contains full bibliography), *Mem. Inst. Oswaldo Cruz*, **22**, 37.
- MALMSTEN, 1848, *Arch. Anat. Physiol.*, Leipzig, p. 1, cited from Sabouraud, R., 1910.
- MATRUCHOT, L. & DASSONVILLE, C., 1899, *Bull. Soc. mycol. fr.*, **15**, 240; 1901, *ibid.*, **17**, 123.
- MEDLAR, E. M., 1915, *Mycologia*, **7**, 200.
- NANNIZZI, A., 1926, *Ann. Mycol.*, **24**, 85; 1927, *Atti Acc. Fisiocr.*, Siena, S. 10, **2**, 89.

- OTA, M., 1924, *Ann. Parasit.*, **2**, 34 ; 1926, *ibid.*, **4**, 1 ; 1928, *Champignons parasites de l'homme*, Tokyo.
- OTA, M. & LANGERON, M., 1923, *Ann. Parasit.*, **1**, 305.
- PELÉVINE, A. (Mme) ET TCHERNOGOUBOFF, N., 1927, *Ann. Derm. Syph.*, Paris, **8**, 403.
- POSADAS, A., 1900, *Rev. Chir.*, Paris, **21**, 277.
- REMAK, R., 1845, *Diagnostische und pathogenetische Untersuchungen*, Berlin, **7**, 193.
- REWRIDGE, A., DODGE, C. W. & AYERS, T. T., 1929, *Amer. J. Path.*, **5**, 349.
- ROBERTS, L., 1899, *Brit. Med. J.*, **1**, 13.
- SABOURAUD, R., 1894, *Les trichophyties humaines*, Rueff et Cie, Paris ; 1910, *Les Teignes*, Masson et Cie, Paris ; 1929, *Ann. Parasit.*, **7**, 228.
- SACCARDO, P. A., 1882-, *Sylloge Fungorum*.
- SARTORY, A., 1919-23, *Champignons parasites de l'homme et des animaux*, Paris.
- SCHENK, 1898, *Johns Hopk. Hosp. Bull.*, **9**, 286.
- SCHOENLEIN, 1839, *Arch. Anat. Physiol.*, Leipzig, p. 82, cited from Sabouraud, R., 1910.
- SHEAR, C. L., 1922, *Mycologia*, **14**, 239.
- TATE, P., 1929¹, *Biol. Rev.*, **4**, 41 ; 1929², *Parasitology*, **21**, 31.
- THOM, C., 1930, *The Penicilli*.
- THOM, C. & CHURCH, M. B., 1926, *The Aspergilli*.
- TULASNE, L. R., 1854, *C.R. Acad. Sci.*, Paris, **38**, 761.
- VERUJSKY, D., 1887, *Ann. Inst. Pasteur*, **1**, 369.
- VUILLEMIN, P., 1898, *C.R. Acad. Sci.*, Paris, **127**, 630 ; 1902, *Arch. Parasit.*, Paris, **5**, 38 ; 1903, *Bull. Soc. mycol. Fr.*, **19**, 119 ; 1910, *Bull. Soc. Sci. Nancy*, **11**, 129 ; 1911¹, *Bull. Soc. bot. fr.*, 166 ; 1911², *Bull. Soc. Sci. Nancy*, **12**, 151 ; 1925, *C.R. Acad. Sci.*, Paris, **180**, 102.
- WEIDMAN, F. D., 1926, *Arch. Derm. Syph.*, N.Y., **13**, 374.
- WERNICKE, R., 1892, *Zbl. Bakt.*, **12**, 159.
- WILENCZYK, M. A., 1927, *Ann. Inst. Pasteur*, **41**, 1351 ; 1928, *C.R. Soc. Biol.*, Paris, **98**, 1049 ; 1929, *ibid.*, **102**, 113.

CHAPTER II. ACTINOMYCOSIS AND SIMILAR CONDITIONS.

BY W. BULLOCH (LONDON HOSPITAL), LEONARD COLEBROOK (ST. MARY'S HOSPITAL, LONDON), F. GRIFFITH (MINISTRY OF HEALTH) AND R. ST. JOHN-BROOKS (LISTER INSTITUTE, LONDON).

History of Actinomycosis.

BY W. BULLOCH.

THE first effective account of this disease was given by O. Bollinger (1877) in Munich. In a series of morbid processes connected with the jaws and throat of cattle and which had been described as osteosarcoma, bone cancer, and wooden tongue, he showed that the lesions were essentially granulomatous in character, and, in addition to granulation cells, leucocytes and detritus, contained numbers of opaque pale yellow, coarsely granular bodies, which on closer examination he regarded as true fungi. This view was confirmed by C. O. Harz (1877-8), a botanist to whom Bollinger submitted some of the fresh material. He regarded it as a new fungus, which from its radial arrangement he called 'Rayfungus' or *Actinomyces bovis*. Independently of Bollinger, James Israël (1878) recognized a similar fungus in a human case, diagnosed as chronic pyæmia, with a large thoracic abscess with purulent metastasis. Israël also gave a detailed description of the fungus. Ponfick (1879, 1882) drew attention to the similarity of the findings of Bollinger and J. Israël, and considered that they represented one and the same disease—actinomycosis. It cannot be asserted, however, that even to-day this has been conclusively demonstrated. It is now known that actinomycotic conditions had been seen in man even before Bollinger and Israël, by Langenbeck in 1845, whose case is described by Israël (1878), and by Lebert in 1846 (published 1857), who clearly recognized the sulphur yellow granules which contained elements 'durs, étroits, cunéiformes, disposé d'une manière rayonnante'. He could not, however, determine their nature. Davaine (1850) also described similar bodies in lesions of the jaws of cattle. Priority claims to the discovery of actinomycosis have also been put forward by Rivolta (1882) and Perroncito (1879). It was, however, the work of Bollinger and J. Israël which first directed attention to the disease, on which there soon accumulated a very large literature (Illich, 1892). Attempts to cultivate the fungus were at first negative, but success was claimed by Oscar Israël (1884) and Bostroem (1890). The organisms isolated by both these observers grew on the surface of the media employed, and Bostroem was largely responsible for the view that the actinomyces is a fungus widely spread in nature and occasionally producing disease in man. On the other hand, Wolff and James Israël (1891) found that their successful cultures were more or less anaerobic,

a view which has been taken by many of the most modern workers, beginning with J. Homer Wright (1904-5) in his elaborate memoir. It cannot be said, however, that the bacteriology of actinomycosis has been completely cleared up, and there still remains a good deal of confusion as to the relation of actinomyces to other fungi of a somewhat similar kind, or even to bacillary forms described by Lignières and Spitz (1902).

The Aerobic Actinomyces.

BY R. ST. JOHN-BROOKS.

NOMENCLATURE.

The genus *Actinomyces* was erected by Harz in 1877, the name *Actinomyces bovis* being applied by him to a branched filamentous organism present in granules secured from pus from cattle affected by 'lumpy jaw'. The fact that the generic name *Actinomyce* was used by Meyen in 1827 for a genus of fungi of quite different characters does not invalidate the use of *Actinomyces* (Buchanan, 1925). The generic name *Streptothrix* Cohn, 1875, is held by some authorities to have priority over *Actinomyces*, but as Corda (1839) had previously given this name to an entirely different fungus, according to the international rule of priority, Cohn's designation is inadmissible. The names *Oospora* Wallroth, 1831, and *Discomyces* Rivolta, 1878, are for various reasons discussed by Buchanan, also invalid. If all the members of the group are to be included in a single genus, it should be *Actinomyces*; if the anaerobic sporeless forms are to be separated from the aerobic conidia bearers, the latter may be included in the genus *Nocardia* Trevisan, 1889, as Wright (1904-5) and Pinoy (1913) think they should. Following, however, the opinion of Lieske (1921), Bergey (1923), Waksman (1927), Lehmann and Neumann (1927), Ford (1927) and Topley and Wilson (1929), it is proposed to regard all the members of the group as belonging to a single genus—*Actinomyces* Harz, 1877. If this be accepted, then, as a natural corollary, lesions in man and animals caused by members of the genus logically should be described as 'Actinomycoses'.

ECOLOGY.

The Actinomyces, or 'ray fungi', are a group of organisms widely spread and present in great abundance in nature, particularly in the soil, representing upwards of 60 per cent. of colonies which develop on agar plates inoculated with soil. They there take an active part in the decomposition of organic matter, both of a nitrogenous and non-nitrogenous nature, and are of considerable importance in the decomposition of 'humus' and the liberation of soil nitrogen. Most members of the genus are purely saprophytic, but some are capable of causing plant diseases, of which potato scab and beet scab (both caused by *A. scabies*) are the best known. Others may enter into a symbiotic relationship

with warm-blooded animals, and may induce infective processes. The most important of these is that caused by the micro-aerophilic species—*A. bovis*—described by Wolff and Israël (see p. 78). But in addition, numbers of various aerobic species have been cited in the literature in association with pathological conditions in man and animals (Actinomycosis, Streptotrichosis, Nocardia and Discomyces infections).

PRINCIPAL CHARACTERS.

The genus is characterized by the formation of a monocellular mycelium composed of slender hyphæ (0.5μ to 0.8μ in diameter), which show true branching, as in the higher fungi. The mycelium develops either in the form of a substrate or as an aerial growth. Owing to the great frangibility of the threads, the mycelial formation is best studied in hanging-drop preparations. All Actinomyces are non-motile and Gram-positive, and many are in small degree acid-, but not alcohol-, fast, especially the aerial spores and hyphæ. The acid-fastness, when present, may as a rule be retained by subculturing on media containing fat, but when once lost cannot be acquired anew. Many species liquefy gelatin and many of the saprophytic strains are strongly hæmolytic. A large number of species form compact gristly or leathery colonies on the surface of solid media, to which the colonies are closely adherent. Many strains form white chalky, round or cylindrical, aerial spores, the cultures, which have a strong smell of fresh earth, appearing as if dusted with chalk. The colonies are frequently brightly coloured—red, yellow, black, green or violet—and the medium may be darkened by a diffusible pigment. The optimum growth for most saprophytic forms takes place at temperatures from 13 to 32° C., but many of the pathogenic forms grow well at 37° C. Some species are thermophilic (Gilbert, 1904; Schütze, 1908).

The Actinomyces are closely related to the Mycobacteria, to which they show all forms of transition. Some species of Actinomyces are non-adherent to the culture medium and are devoid of aerial hyphæ, while some strains of Mycobacteria show ramifying forms in old cultures which greatly resemble ray fungi. The Actinomyces exhibit in their morphological and physiological peculiarities a variability which is scarcely possessed by any other group of organisms: for this reason they are very difficult to classify.

INFECTIONS DUE TO OR ASSOCIATED WITH AEROBIC ACTINOMYCES.

Mycetoma.

Mycetoma is a term applied to the invasion of the foot ('Madura foot'), hand and more rarely of other parts of the body, by fungi belonging to the Actinomyces (and other genera) which produce enlargement, deformity and destruction of the affected parts. The lesions are of a chronic granulomatous nature, characterized by swelling, suppuration and sinus formation. They are associated with granules of aggregated

mycelial filaments, varying in colour, form and size, generally eliminated exteriorly by means of more or less well-defined fistulæ. The presence of definite grains separates the mycetomas from pseudo-mycetomatous conditions (sporotrichosis, &c.).

The mycetomas have been divided by Pinoy (1913) into two classes, the 'actinomycoses' and the 'true mycetomas'. The latter are described by Chalmers and Archibald (1916) as 'maduromycoses', with which classification Langeron (1922) is in agreement.

The two classes are defined as follows :

The *actinomycoses* are those forms of mycetoma with grains composed of very fine non-segmented mycelial filaments, in which usually the walls are not clearly defined from the contents and in which chlamydospores are absent.

The *maduromycoses* are those forms of mycetoma with grains composed of large segmented mycelial filaments possessing well-defined walls and usually chlamydospores.

The term 'Madura foot' is synonymous with mycetoma of the foot and should only be used for mycetomas in this situation. In 1860 Vandyke Carter of Bombay applied the term 'mycetoma' to that variety of Madura foot disease which contained black granules, and, a year later, included under this name the yellow or white variety of the same complaint. These two forms, described by Carter, correspond respectively to the two groups above defined.

The Actinomycoses.

The mycetomas, due to actinomycotic infections, may be divided into two sections, the first comprising the anaerobic forms, including Wolff and Israël's organism (see p. 78), the second, with which we are here concerned, the aerobic organisms.

Actinomyces maduræ (Vincent) Lehmann and Neumann [*Nocardia indica*, Kanthack (1893), Carter's or Vincent's White Mycetoma]. Probably the most widespread form of mycetoma in warm countries is caused by this fungus, associated with an ochroid or white form of Madura foot. It was first cultivated by Vincent (1894), and called by him *Streptothrix maduræ*. Cases have been reported from India, Northern Africa, North and South America, and Southern Europe. A strain isolated by Welchman and Harvey Pirie (1921) from 'Madura foot' in a South African native is conserved at the National Collection of Type Cultures (1925) (N.C.T.C. No. 1070).

The grains consist of slender interwoven filaments with a tendency to a radiating arrangement. Small irregular swellings, but not 'clubs' are often seen at the periphery.

The organism, which is a strict aerobe, grows best at 37° C., but also well at temperatures between 20 and 40° C. Growth in broth is poor, forming a greyish white, granular 'puff-ball' deposit, without turbidity. On agar slope the growth after seven days consists of scanty, greyish

white, opaque heaped-up colonies with nodular surfaces. Raised, nodular, yellowish brown colonies are formed on potato after seven days' incubation at 37° C. In older cultures these colonies turn bright orange red or deep red, and may be covered with a fine white dust consisting of spores. Milk is not coagulated and gelatin is not liquefied, or very slowly so. Microscopically, the growth consists of long, branching non-segmented filaments, which are Gram-positive and not acid-fast. The organism is very resistant to drying, but non-sporing cultures are killed in from 3 to 5 minutes by moist heat at 60° C. Subcutaneous inoculation into rabbits, mice and cats causes nodule formation at the seat of inoculation, which, after a time, retrogresses.

Actinomyces asteroides (Eppinger) Gasperini (Musgrave's and Clegg's White Mycetoma). Another widely spread form of whitegrained mycetoma is that produced by *A. asteroides*, first isolated by Eppinger (1891) from a cerebral abscess and from pseudo-tubercular lesions in the lungs and pleura. Numerous cases of mycetomas caused by this organism have since been reported from Europe, North and South America, Asia and Africa. The *Streptothrix freeri* Musgrave and Clegg (1907) from a case of mycetoma with yellowish grains from the Philippines and the *Discomyces brasiliensis* of Lindenberg (mycetoma of leg) represent the same organisms. The grains, which are composed of Gram-positive, acid-fast filaments, do not contain 'clubs'. On agar are formed star-shaped, dry, wrinkled colonies, at first whitish and later red. Gelatin or inspissated serum is not liquefied. The growth on agar or potato varies in colour from orange yellow to brick red. *A. asteroides* is pathogenic to monkeys, rabbits and guinea-pigs—small white nodules and abscesses in the viscera resulting from subcutaneous inoculation.

Actinomyces somaliensis (*Discomyces somaliensis* Brumpt, 1913). (Bouffard's White Mycetoma.) This condition has been observed by Bouffard in French Somaliland, by Balfour (1911) in the Anglo-Egyptian Sudan (mycetoma of hand), by Fülleborn (1911) in German South-West Africa, and by Chalmers and Christopherson (1916) in the Sudan. It is characterized by the complete destruction of the bones involved. The grains, which are about 1 mm. in diameter, are easily recognized by their resemblance to fish-roe, by their reddish colour and their extreme hardness (insoluble in potash). Club formation is absent. The colonies on potato remain yellowish and do not take on a red coloration as do those of *A. maduræ*. This organism was at first placed by Brumpt (1906) in the genus *Indiella*.

Actinomyces convolutus [*Nocardia convoluta* Chalmers and Christopherson (1916), Chalmers and Christopherson's Sudanese Mycetoma]. Isolated by Chalmers and Christopherson from a mycetoma of the foot observed at Khartoum, which formed a fibrous tumour without fistulæ. The grains, formed of fine branched, Gram-positive non-acid-fast filaments enclosed in a more or less homogeneous matrix, are small (0.5 mm.), soft, smooth and orange coloured. Clubs are not present. The organism does not

liquefy gelatin. It grows best on solidified blood-serum at 30° C., forming light buff, convoluted, raised colonies (hence the name—'*convoluta*'). The medium, which is eventually liquefied, is not coloured. *A. convolutus* is apparently non-pathogenic to monkeys, white rats, gerbils, rabbits and pigeons.

Actinomyces pelletieri [*Micrococcus pelletieri* Laveran (1906), Pelletier's Red Mycetoma]. First reported by Pelletier, who described a case of mycetoma with red grains that he saw in Saint-Louis, Senegal, where the condition is apparently fairly common (Thiroux and Pelletier, 1912). The grains were very small (0.4 mm. to 0.5 mm. in diameter), and of a beautiful vermilion colour. They contained numerous Gram-positive 'micrococcal' bodies embedded in a homogeneous ground substance. In the parasitic stage the organism takes the form of a micrococcus in zooglea. Growths on solid media do not penetrate the surface, and, unlike *A. maduræ*, are red from their commencement (N.C.T.C. No. 3026).

According to Pijper and Pullinger (1927) the mycetomas met with in South Africa appear to be always of actinomycotic origin. The Maduromycoses have not up to the present been met with in the sub-continent. They describe three new species of *Actinomyces* isolated from cases of mycetoma in South African natives, which they have designated as *Nocardia pretoriana*, *N. transvalensis* and *N. africana* respectively (N.C.T.C. Nos. 2891, 2892 and 2893 respectively).

Cornwall and Lafrenais (1922) have recorded a new species of *Actinomyces* (*Streptothrix*) from mycetoma of the leg in India which differs from all other published descriptions.

The Maduromycoses.

According to Brumpt (1906) and others, a considerable number of fungus species other than *Actinomyces* may be implicated as causal agents of mycetomas. For example, 'Nicolle's white mycetoma' is associated with the ascomycete—*Aspergillus nidulans* Eidam, while other forms of the white or ochraceous variety are due to invasions of species of *Indiella* Brumpt, *I. mansonii* Brumpt and *I. reynieri* Brumpt, being associated respectively with Brumpt's and Reynier's white mycetomas. Doubtless many other forms exist, such as *Scedosporium apiospermum* Saccardo, which has been isolated several times in Italy and Sardinia and recently in Brazil (da Fonseca and Leão, 1927) from clinical cases of 'Madura foot' disease (N.C.T.C. No. 2768).

The melanotic or black varieties of the disease are due to quite different organisms. 'Carter's black mycetoma', to which reference has already been made, was investigated by Laveran (1902), and from the lesions an organism—the *Madurella mycetomi* of Brumpt was isolated. Another species of this genus—*M. tozeuri* (Nicolle and Pinoy, 1908)—has been isolated from cases of Nicolle and Pinoy's black mycetoma from Tunis. A full account of the black mycetomas is given by Chalmers and Archibald (1916), who describe a new Sudanese variety caused by the fungus

Glenospora khartoumensis Chalmers and Archibald, nearly allied to, but not identical with, the fungus grown by Semon (1915) from a case of Indian maduromycosis.

Cattle Farcy. Farcin de Bœuf.

[*Actinomyces farcinicus* (Trev. and de Toni) Gasparini.]

The causal organism of 'Farcin de bœuf' was originally isolated by Nocard (1888) from cattle disease in Guadeloupe characterized by chronic suppurative inflammation of the superficial lymphatic glands with abscess and sinus formation followed by pulmonary and visceral lesions. *A. farcinicus*, which is probably present in stable litter and soil, is thought to be transmitted, in Guadeloupe, by a tick of the family *Ixodidae* (*Hyalomma aegyptium*). The disease is not common in Europe, but has been reported from India. Bovine farcy only affects cattle and must be distinguished from farcy in man and horses due to *Bacillus mallei* (see Vol. V).

The fungus occurs in the pus and affected tissues in the form of delicate twisted filaments with numerous peripheral burr-like prolongations. 'Clubs' are absent. The organism, which is strictly aerobic, Gram-positive and not acid-fast, or feebly so, grows readily on ordinary laboratory media at from 30 to 40° C., forming on agar, small, irregular, mammilated, yellowish white, dull and powdery colonies, which later coalesce to form a coarsely pleated membrane. Rapid growth takes place on potato, with formation of dry, pale yellow plaques with sharply cut edges. Whitish, irregular, flocculent growth occurs in broth, either floating on the surface or falling to the bottom, the medium remaining clear. In cultures the organism forms masses of tangled filaments with bacillary structure. Old cultures are rich in small oval spores. There is no liquefaction of gelatin or change in litmus milk.

Vitality and virulence remain for a long time under artificial conditions, virulence for the guinea-pig being retained after four months at 40° C. The organism is also virulent to sheep and cattle by intravenous or intraperitoneal inoculation, giving rise to miliary nodules in the peritoneum, kidney, spleen, &c. Rabbits, horses, asses and dogs are resistant to such inoculations.

Other Actinomycotic Infections of Man and Animals.

Numerous cases are on record of *Actinomyces* of the aerobic type having been found in lesions, other than definite mycetomas, of man and animals. Among the more important strains mentioned in the literature may be mentioned the *Streptothrix* of Birt and Leishman (1902), an acid- and alcohol-fast organism isolated from pleural fluid and sputum from a fatal case of pseudo-tuberculosis (N.C.T.C. No. 658), *Nocardia lutea* (Christopherson and Archibald, 1918), isolated from disease of the lachrymal gland at Khartoum (N.C.T.C. No. 576) and *Actinomyces gypsoides* isolated from sputum by Henrici and Gardner (1921). An

aerobic non-acid-fast *Actinomyces* (N.C.T.C. No. 600), unassociated with the anaerobic form, was isolated in pure culture by the writer in 1919 from pus from a deep-seated parotid abscess opened with surgical precautions. Smears from yellowish granules contained in the pus showed ramifying masses of Gram-positive filaments with club-shaped forms at the free ends. Silberschmidt (1899) described a form—*Actinomyces* (*Streptothrix*) *caprae*—isolated from a goat affected with pseudo-tuberculosis (N.C.T.C. No. 659) and Mason (1919) has isolated another species—‘*Streptothrix cameli*’ from the lung of a camel with generalized actinomycosis. Among other strains conserved in the National Collection of Type Cultures are a ‘*Streptothrix*’ isolated from ‘joint-ill’ in a foal (N.C.T.C. No. 630) and ‘*Nocardia*’ species isolated from guinea-pigs and rabbits in Sumatra, described as ‘*N. caviae*’ (N.C.T.C. No. 1934) and ‘*N. cuniculi*’ (N.C.T.C. No. 1935) respectively.

SYSTEMATIC POSITION OF GENUS *ACTINOMYCES*.

The systematic position of the group as a whole demands much further enquiry. There has been a tendency in the past to attach, often on inadequate investigation, specific names to organisms of this class associated with particular lesions, without much regard to claims of comparative taxonomy. With the object of aiding further systematic studies of this difficult group, the writer would emphasize the desirability of forwarding strains to the National Collection of Type Cultures, where they would be at the disposal of those interested.

The Anaerobic Actinomyces.

BY L. COLEBROOK.

Under this heading it is proposed to deal only with the anaerobic mycelial organism, *Actinomyces bovis*, first described by Wolff and Israël (1891), and afterwards more fully by Homer Wright (1905). Cultural investigations of the pyogenic mycelial infections occurring in different parts of the world have led to the conclusion that this organism is by far the most common infective agent in the group of cases characterized by a granulomatous lesion productive of pus in which are definite ‘sulphur granules’. To avoid confusion it would therefore seem desirable that the term ‘actinomycosis’ should in future be employed to connote only infections by this one bacterial type. That recommendation, if followed strictly, would entail the cultivation and study of the organism from every case before a diagnosis of actinomycosis was definitely registered. In practice, however, such cultivation is not absolutely necessary because, just as in tuberculosis the demonstration of acid-fast bacilli is sufficient for a diagnosis which will be correct ninety-nine times out of a hundred, so in actinomycosis the finding of definite granules visible to the naked eye and composed of Gram-staining mycelium will make it legitimate to infer the probable presence of an infection by *Actinomyces bovis*.

It would be going too far to state, as some have done, that the anaerobic type here denoted '*Actinomyces bovis*' is the only mycelial organism capable of producing such 'sulphur granules' in the body, but there can be little doubt that in most parts of the world such granule colonies are *usually* composed of that microbe and hence it follows that a clinical diagnosis based upon the finding of these granules will in most instances be justified. The evidence upon this point may be summarized as follows : Homer Wright (1905) in America isolated this organism from 13 consecutive cases showing such granules ; Shiota in Japan (1909) in 28 cases out of the 29 from which a pure culture was obtained ; Schukewitsch (1903) in Russia from 22 cases out of 25 investigated ; Harbitz and Gröndahl and their colleagues (1911), in Norway, from 20 cases (number investigated not stated) ; and Colebrook (1921) from 21 cases out of 24 investigated.

The infection of tropical countries known as Madura foot or mycetoma appears to provide an exception to the generalization that mycelial granules are usually to be correlated with the presence of *Actinomyces bovis*. At any rate those mycetoma cases which are productive of black or red granules are almost certainly infected by a different organism. Those with white granules may prove to be infected by *Actinomyces bovis*, but that has yet to be established.

The Characters of Actinomyces bovis.

It is recognized that small differences occur among a number of strains, but the following account represents the writer's experience of the majority :

Morphology. This organism is a slender, non-septate mycelium (0.5 to 1.0 μ in diameter) showing true branching. (This has been particularly well shown by cultivating the organism in a thin blood-clot between two microscopic slides as described by Sir A. E. Wright in connection with tubercle bacilli.) On artificial culture medium (glucose agar) the growth is sometimes more bacillary than mycelial. Spores have not been recognized, nor the spore-bearing septate hyphæ which Dreschler described in connection with the aerobic *Actinomyces* of soil.

The 'sulphur granules' produced by growth *in vivo* have the form of a cauliflower head, being compounded of a number of minute masses of compact mycelial feltwork, each sharply delimited externally by a radial arrangement of the characteristic 'clubs'. These 'clubs' are pear-shaped thickenings on the terminal filaments of mycelium. Their formation *in vitro* has been reported by Homer Wright (1905), who observed them in cultures of the organism grown in pleural exudate. More recently, Bayne-Jones (1925) has succeeded in detecting them in paraffin-embedded sections prepared from colonies grown in glucose broth. By the continuous observation of cultures in a shallow moist chamber he was also able to detect the growth of new filaments from the narrow end of the clubs. Filaments up to 100 μ in length, with branches, were seen to arise in this manner.

Staining reactions. The mycelium of 'granules' retains Gram's stain, but not uniformly, so that each thread presents darkly and lightly stained sections. That of cultures on artificial media is usually more deeply and somewhat more uniformly stained. It is not acid-fast. The clubs of the fresh granules are best seen by direct examination (unstained) under a cover-glass with a narrow diaphragm. They are not easily stained differentially in the fresh, unfixed, state. In sections of tissue they are well displayed by eosin or acid fuchsine following iron hæmatoxylin.

Cultural characters. Broadly speaking, *Actinomyces bovis* is an obligate anaerobe, although not a very strict one, inasmuch as after acclimatization to artificial media a sparse growth can sometimes be obtained under aerobic conditions. Magnusson (1928) states that it grows readily in an atmosphere of CO₂. In shake cultures (glucose agar) the zone of maximum growth is usually about 1 to 1.5 cm. below the surface and very little growth occurs above that level. The colonies in such cultures are readily distinguishable from most of the common bacterial types by virtue of their irregular cauliflower-like surface and dull white colour. Surface cultures are most easily obtained in the McIntosh and Fildes (1916) anaerobic jar (palladium chloride method). The character of these colonies is much the same as those of shake cultures, viz. white, opaque and of irregular surface. If examined in the early stages of growth with a low magnification they often show radial offshoots, rather suggestive of the medusa-head colony processes of *B. sporogenes*. The colonies are somewhat adherent to the medium and are not very easily broken up. Good photographs of surface growths are to be found in the paper of Wolff and Israël. On blood agar and egg medium the character of the growth is very similar to that on glucose agar. Growth in fluid media (glucose broth) occurs in the form of compact breadcrumb-like masses at the bottom of the tube, the broth above remaining perfectly clear. In human serum growth occurs only if the antitryptic power has been neutralized. The optimum temperature for growth is about 37° C. No growth is obtained at 22° C. The optimum pH of the medium is 7.3 to 7.6.

Viability. Homer Wright and others have reported that the mycelium of granules may be kept alive in the ice-chest for several weeks. Pure cultures do not live as a rule more than 10 to 14 days, but on Dorset's egg medium they will sometimes survive for 3 to 4 weeks in the ice-chest. Heating to 60° C. has always killed them.

Serological reactions. Colebrook (1920) has reported that the serum of heavily infected patients will agglutinate suspensions of *Actinomyces bovis*, and that preliminary observations with specific agglutinating rabbit sera point to the existence of distinct serological types.

Pathogenicity of Actinomyces bovis.

Inoculation of animals. Wolff and Israël inoculated pieces of agar culture into rabbits and guinea-pigs, usually into the peritoneum. Many of the animals, when killed a few weeks or months later, showed circumscribed

tumours of varying size, containing cheesy material. In sections of these tumours colonies of *Actinomyces* were found with peripheral clubs. Cultivation of the organism from the lesions was sometimes successful. Most of those who have repeated these experiments have obtained less definite results. Thus, Homer Wright (who reports his experiments in detail and also summarizes those of other workers) usually obtained only minute non-progressive foci of infection, sometimes with a little caseous material. He was not satisfied that there had been actual multiplication of the organism after its introduction into the animal body.

During the last few years this question has been taken up again by two Swedish bacteriologists, working independently. Naeslund (1929), in Upsala, has reported on a number of inoculations of cattle as well as smaller animals. Five pure cultures derived from human actinomycosis were employed. In pigs and guinea-pigs no lesions were produced. In the peritoneum of rabbits, circumscribed tumours, as large as a pea, were found, with cheesy contents, which did not, however, show any 'sulphur granules'. *Actinomyces* were not recovered in cultures from this material. Inoculations into the anterior chamber of rabbits' eyes resulted in some multiplication of the mycelium, and, in one instance, when the rabbit was killed after twenty-seven days, some thickening and infiltration of the iris was found. Naeslund did not regard this as an unquestionable production of an actinomycotic lesion. Of five cattle which were inoculated subcutaneously, four developed no lesions. In the fifth—an eight months heifer—lesions began to develop at two of the sites of inoculation after three weeks. Both of these progressed, with suppuration, until they were excised six months later. The one produced a polypoid growth, the size of a hen's egg, with foci of softening from which evil-smelling pus was discharged. The other extended beneath the skin till it was as large as the palm of the hand, and from its softened centre a fistulous opening developed, discharging 'sulphur granules' with clubs. *Actinomyces* were recovered in pure culture from these granules.

Magnusson, a veterinary bacteriologist working in Malmö, has also reported (1928) the production of 'actinomycomas' in cattle by the subcutaneous inoculation of pure cultures of *Actinomyces bovis* (Wolff-Israel type) derived from actinomycotic lesions of cattle. One of his strains inoculated into two young cattle produced tumours as large as a walnut in both animals after sixteen days. The other strain infected much more slowly, but after 3 to 4 months, 4 of the 6 cattle inoculated showed 'local reactions' which developed into 'fistulæ and granulomatous vegetations, exactly as in spontaneous actinomycosis'. In all four cattle these vegetations, in their later stages, were described as polypoid and having the size of a hen's egg. On section they showed 'granulous pus' 'focally sprinkled in half-elastic granulation tissue'. Pure cultures of *Actinomyces* were obtained from this pus.

Spontaneous infections of cattle. For a full and critical account of these, Magnusson's recent paper (in English) should be consulted. His own

work, and that of Hülphers in Stockholm and Gunst in Amsterdam, show that the great majority of *Actinomyces bovis* infections of cattle are confined to the jaw and adjacent soft parts of the head and neck. They are usually purely local lesions, which form one or several fistulæ, and discharge pus. When, occasionally, dissemination does take place, it is *not* by the lymphatic channels (as is the case in actinobacillosis of the tongue) but by the blood-stream. Granules from these cattle lesions, when crushed between slides and stained by Gram's method, show 'an exceptionally varied picture of all imaginable forms of bacteria', and yet Magnusson experienced no difficulty in obtaining pure cultures of *Actinomyces* from them. This statement very strongly suggests to the present writer that the highly pleomorphic *B. actinomycetem comitans* was present along with *Actinomyces* in the granules. It would be almost impossible to detect the minute transparent colonies of that organism on the Loeffler's serum medium which Magnusson usually employed for his cultivations. The strains of *Actinomyces* which he isolated from 48 of the 61 granulomatous lesions occurring about the jaws of cattle resembled 'in all essentials' the anaerobic species described by Wolff and Israël, Homer Wright, Colebrook and others.

Infections of man. If we exclude cases of mycetoma (Madura foot), actinomycotic lesions of man are found almost invariably in one of three situations, viz. the cervico-facial region, the right iliac fossa, and the thorax. Being comparatively painless they usually first attract attention by the formation of an abscess under the skin, and after this has been evacuated, run a chronic course, often discharging by several sinuses. The typical granules may often be detected on the somewhat abundant granulations at the mouth of the sinuses. It is characteristic of these lesions that they extend by direct spread in the connective tissues and very rarely by the lymphatic or blood channels; they are very sharply circumscribed; and, *unlike the infections by Actinobacillus*, very seldom involve lymphatic glands.

The cervico-facial cases usually recover when the pus is given free drainage. The thoracic cases, on the other hand, usually die, probably because it is difficult to drain them freely. As to those which originate in the right iliac fossa as a suppurative appendicitis it is difficult to speak with any certainty, for it has been suggested (Slesinger, 1920) that these cases may occur much more frequently than is commonly recognized, and although many of them certainly die, others may be promptly cured by drainage of the abscess.

The Ætiology of Actinomycosis.

The long-accepted view of Bostroem (1891) and others that the infective agent of actinomycosis is a common parasite of the vegetable world, living particularly on cereals, grasses, &c., and that it is conveyed by these materials to the human or animal host, is now regarded as unproven and somewhat improbable. Certainly cases have occurred from time to time

in which a foreign body of vegetable nature (a blade of grass, a head of barley, &c.) has been evacuated from an abscess cavity along with mycelial organisms, but there is reason to think that some of these cases were not true actinomycotic infections in the sense in which the term is here employed—one such case has recently come to the writer's notice—and with regard to all of them it has to be remembered that the organism might have been derived from the patient's own mouth or intestine and found its opportunity to invade the tissues because these had been injured by the impacted foreign body.

The essential point of evidence in favour of the exogenous hypothesis has never been produced, viz. the evidence that *Actinomyces bovis* does exist as a parasite of the vegetable world, and it seems unlikely that this evidence will ever be forthcoming, for the organism is now well recognized to be a very delicate anaerobic microbe, which grows only at body temperature and is incapable, so far as we know, of forming resistant spores. For fuller discussion of this question the reader is referred to the papers of Homer Wright (1905) and Colebrook (1921).

The alternative hypothesis presupposes that *Actinomyces bovis* is frequently present in the alimentary tract, but is able to invade the tissues only under specially favourable circumstances. This view also lacks definite proof at present, but it has gained some support both from clinical observation and from experimental work. Of the clinical records which afford support to it perhaps the most striking is that of the case reported by Israël (1887), in which a fragment of carious tooth—identified by section—was found *post mortem* in an actinomycotic focus of the lung; and that of Cope's case (1915) who developed actinomycosis on the knuckles of the right hand following injury by an adversary's broken tooth in a street fight. The case recently reported by Judd, in which actinomycosis followed directly upon extraction of a tooth, is also instructive in this connection.

The chief experimental evidence is that furnished by Lord (1910) and Naeslund. Lord called attention, as others had done previously, to the frequent presence of thread-like organisms resembling *Actinomyces* in the contents of carious tooth cavities and of tonsillar crypts, and although he did not isolate these organisms in pure culture he reported that the inoculation of the material from such teeth and tonsils into the peritoneum of guinea-pigs sometimes resulted in the development of small suppurative lesions having the histological characters which are associated with actinomycosis. Naeslund's work (1925 and 1926) has gone much farther. He has succeeded in isolating both aerobic and anaerobic strains of *Actinomyces* from the human mouth, and states that some of his anaerobic strains appear to be identical with *Actinomyces bovis* derived from cases of actinomycosis. He also claims to have produced (occasionally) an actinomycotic tumour containing club-bearing granules, by the sub-cutaneous inoculation of cattle with material taken direct from the surface of human teeth [personal communication (1929)—unpublished].

In his later work, Naeslund (1926) has studied the relation of the various types of *Actinomyces*—and also *Leptothrix*—to the formation of tartar in the mouth. From his experimental work he concludes that these organisms, by their growth in the saliva and serous exudates of the gums, bring about a decrease in the hydrogen-ion concentration and 'decomposition of the colloid proteins', which together result in precipitation of lime-salts. The filamentous growth of the *Actinomyces* and *Leptothrix* on the teeth affords a suitable framework for the deposition of these salts.

If this view of the formation of tartar deposits is confirmed, and if it can be shown that *Actinomyces bovis* is sometimes incorporated in these deposits, there will be no need to look elsewhere for the source of the infecting agent of actinomycosis. Fragments of tartar, or of tartar-encrusted teeth, must be very frequently detached in the mouth, and it is not difficult to suppose that on rare occasions one of these, becoming impacted in the tissues, either of the mouth or the alimentary or the respiratory tract, will set up a slowly progressive inflammation.

The Detection and Isolation of Actinomyces bovis.

Random samples of pus should never be employed for the preparation of films, or for cultivation, if the presence of *Actinomyces* is suspected. This organism is almost always confined to the 'granules', and unless one of these is by chance included in the random sample, the nature of the infection is likely to be missed. Granules may often be found by spreading out the pus thinly on the side of a test-tube, or on a Petri dish: where this fails they may be detected by shaking a few drops of the pus vigorously in physiological salt solution. (This is conveniently done by means of a simple mechanical shaker of the type figured in 'The Teat and Capillary Glass Tube', Wright and Colebrook, 1921.) All the other elements of the pus will usually break up under violent shaking, and the actinomycotic granules can then be seen sinking to the bottom of the tube. From this they are fished out by a capillary pipette and transferred to a microscopic slide for direct examination, or stored in broth for the inoculation of suitable culture media.

The direct examination of suspected granules should consist of: (a) examination unstained under a cover-glass lightly pressed down—and with restricted lighting—in order to detect the peripheral fringe of clubs and the characteristic cauliflower structure of the granule; (b) examination of a Gram-stained film made from a more completely crushed granule. This will reveal the characteristic slender mycelium, and also, if fuchsin has been used for the counterstain, will usually show the presence of *B. actinomycetem comitans*.

For cultivation a number of granules are lightly crushed between two sterile microscopic slides with a few drops of broth. The coarse suspension so obtained is then planted direct, or when secondary infections are likely

to be present, after suitable dilution, as follows: (1) In glucose broth with a drop of blood under a seal of liquid paraffin (Gordon, M. H., 1920). Whole granules and a drop of suspension should be planted. (2) In glucose agar shake cultures under vaseline. These are very conveniently made in pipette tubing. The relatively narrow column of medium facilitates the examination and removal of individual colonies. (3) On glucose agar plates in the McIntosh and Fildes anaerobic jar (1916).

Further Work required in connection with Actinomycosis and Allied Infections.

(1) To establish or refute the suggestion that *Actinomyces bovis* is frequently present in the human mouth and throat. Isolation of the organism and identification by means of agglutination with specific sera.

(2) Cultural experiments to facilitate the isolation and preservation in pure culture of *Actinomyces bovis* and allied organisms.

(3) To define the different serological types of *Actinomyces bovis*.

(4) To attempt the classification of mycelial organisms recoverable from: (a) the para-actinomycotic infections (Colebrook, 1921), occasionally met with, i.e. infections characterized by the presence of typical granules, but not caused by *Actinomyces bovis*; (b) cases in which the infective agent is a mycelial organism other than *Actinomyces bovis*, and in which the clinical condition more or less resembles actinomycosis, but granules are not found (streptothricosis, nocardia).

(5) To obtain an effective chemotherapeutic remedy for the disease.

(6) On the significance of the association of *B. actinomycetem comitans* with actinomycosis.

BACTERIUM ACTINOMYCETEM COMITANS.

Klinger, in 1912, described under this name a small cocco-bacillary organism which he had met with in four cases of human actinomycosis occurring in Zurich. It was present in great numbers along with *Actinomyces* filaments in the interior of the granules, and apparently did not occur free in the pus. In pure culture it presented one distinguishing characteristic, viz. its habit of adhering to the glass walls of the culture tube. Klinger's findings have been confirmed in all respects by Colebrook (1920), who observed organisms having the morphological characteristics of *B. actinomycetem comitans* in films prepared from crushed actinomycotic granules of 24 out of 30 human cases of actinomycosis occurring in London between 1912 and 1921. Pure cultures were obtained from at least ten of these cases. Bayne-Jones (1925) in America isolated the organism from a closed lesion in a case of human actinomycosis.

Morphology. When an actinomycotic granule is crushed between two microscopic slides the resulting films, stained by Gram's method and counterstained with weak carbol-fuchsine, frequently show the *Actinomyces* filaments (Gram-positive) upon a more or less granular background which

has taken the counterstain. If this background be traced to its edges and carefully examined it will be found to consist of innumerable Gram-negative pleomorphic elements—micrococci intermixed with bacillary forms of 1 to 3μ in length—or sometimes almost entirely of bacillary elements. These are the in-vivo forms of *B. actinomycetem comitans*. When transferred to suitable culture media, crushed granules showing these organisms will sometimes, but not always, yield a multitude of slow-growing (2 to 3 days) pin-point colonies which are composed of pleomorphic elements similar in form and staining reactions to those observed in the granules. The micrococcal forms are decidedly smaller than staphylococci, and, being often slightly elongated, bear some resemblance to *Brucella melitensis*. The bacillary forms have rounded ends. Neither spore nor capsule formation have been demonstrated. The organism is non-motile. It must be remembered that the different morphological types may represent different organisms, but the evidence so far available is not in favour of that view.

Staining reactions. As stated, Gram's stain is not retained. The organism is not acid-fast and is not readily stained by methylene blue or neutral red. A suggestion of bipolar staining is sometimes obtained by Leishman's method.

Cultural properties. The microbe is a facultative anaerobe which will grow on ordinary nutrient media at body temperature but not at 22° C. Enrichment with serum seems to favour growth. Surface colonies somewhat resemble those of *Streptococcus pyogenes* on ordinary agar, but come to maturity more slowly, i.e. in 2 to 3 days. They seldom exceed 1 mm. in diameter, are nearly transparent, discrete and hemispherical. Their outstanding characteristic is the tenacity with which they hold together and cling to the medium on being disturbed. Fluid cultures in liquid gelatin or broth are characterized by the same remarkable tenacity. They grow only in contact with the glass wall of the tube and can be fairly vigorously shaken without being dislodged.

Viability. Klinger reports that agar and gelatin cultures live for 4 weeks. Frequently, however, the survival period is not more than 2 to 3 weeks.

Pathogenicity for laboratory animals. Subcutaneous inoculation of mice produced no effect (Klinger), and in guinea-pigs only a local inflammation (Colebrook). Intravenous inoculation proved rapidly fatal to two rabbits, and a diffuse fatal peritonitis was set up in two guinea-pigs by intraperitoneal inoculation.

On the significance of the association of B. actinomycetem comitans with Actinomyces. The fact that this microbe has not been met with except in association with *Actinomyces bovis* in the granules of human lesions suggests either that there is some genetic relationship between the two species or that actinomycotic lesions are frequently the result of a double infection. These alternatives were discussed by Colebrook (1920), who was, however, unable to arrive at any conclusion.

Actinobacillosis.

BY F. GRIFFITH (MINISTRY OF HEALTH).

The special interest attached to the discovery of the actinobacillus is its bearing on the ætiology of 'actinomycosis'. It had long been held that this disease was identical in man and animals and was due to infection with a branched filamentous organism which gave rise to the characteristic granules in the tissues and purulent discharges. In human actinomycosis these granules invariably exhibited in suitable preparations the typical clubs together with masses of the Gram-staining organism. In bovine actinomycosis, on the other hand, whilst the clubs were a prominent feature of the granules, it was often impossible to demonstrate any elements which retained the Gram stain. There were various interpretations of these irregularities, but Lignières and Spitz, in the Argentine, were the first to reveal the true explanation, which shattered the conception of actinomycosis as a single morbid entity. These writers, who published their classical work in 1902 and 1903, showed that many of the cases of actinomycosis in bovine animals were caused not by a Gram-positive branching organism but by a small Gram-negative bacillus. To this bacillus they gave the name of *Actinobacillus*, and they suggested that the particular form of actinomycosis which it produced should be designated *actinophytose à actinobacille* in contradistinction to the *actinophytose à streptothrix*, which is the less common variety in bovines. It was my experience in 1914-15 that out of 44 cases of actinomycosis of cattle in the British Isles only four belonged to the streptothrix group, and in these four the disease had attacked the bone of the lower jaw.

The results of Lignières and Spitz were confirmed by Nocard (1902), and later by Higgins (1908), Griffith (1916), Magnusson (1922), and others.

Distribution of Actinobacillosis.

The disease, as already stated, was first described in Argentina, where it sometimes occurred in epizootic form. It has now been shown to be widely distributed throughout the world and to be responsible for considerable suffering and loss amongst domestic animals. It affects cattle and swine; in the latter the mammary glands are commonly the seat of the disease. There is no authentic record of a human case.

Descriptions of Lesions.

Actinobacillosis attacks mainly the softer tissues and unlike *Streptothrix actinomyces* rarely infiltrates the bone. Another point of distinction is the frequency with which lymphatic glands are affected. The disease in cattle occurs most commonly in the neighbourhood of the mouth, e.g. woody tongue, and is characterized by extensive overgrowth of fibrous tissue in which the typical granules are embedded. In the lymphatic glands the condition can be recognized on naked-eye inspection; the glands contain soft, yellow or orange-coloured granulomatous tumours

which project above the cut surface and, on being scraped with a knife, yield the tiny opaque granules. In the centre of these the organism appears as masses of minute Gram-negative cocco-bacilli. The clubs surrounding the central mass are the most prominent feature and possibly consist of a bacillary secretion. It might be of interest to ascertain whether the substance of which the clubs are composed is akin to the soluble carbohydrate secreted by the pneumococcus.

The Characters of the Actinobacillus.

The *Actinobacillus* can readily be obtained from fresh intact nodules, and the lesions in the lymphatic glands are particularly suitable for the purpose of culture. It grows well on ordinary nutrient agar, the bluish-grey colonies attaining a diameter of 1.5 mm. after 24 hours at 37° C. On ordinary nutrient agar it appears in the form of a short bacillus with occasional long forms, but in glucose agar shake cultures the rosette-shaped colonies consist of masses of long, tangled, unbranched filaments. The bacillus is non-motile, stains best with weak carbol-fuchsin and is Gram-negative. Its viability in culture is not great. It ferments glucose, maltose, saccharose, xylose and mannitol quickly, lactose and raffinose slowly, and the other sugars not at all (Magnusson, 1928).

Agglutinins are formed in infected animals, and are of assistance in differential diagnosis during the life of the animal, when it may not be possible to obtain pieces of the pathological tissue.

Pathogenicity.

The typical disease can be produced in bovine animals by the injection of pure cultures (Griffith, 1916; Magnusson, 1922). Lignières and Spitz found, in addition, that after intraperitoneal injection of guinea-pigs purulent nodules were formed, in which could be demonstrated the characteristic granules and clubs. This result has not been obtained by other workers, but there are, no doubt, differences in virulence between strains, and it is possible that actinobacilli from the Argentine, where the disease may assume epizootic proportions, have a greater infectivity than European strains.

REFERENCES.

- BALFOUR, A., 1911, *Fourth Report of the Wellcome Tropical Research Laboratories*, A. Medical, p. 365.
 BAYNE-JONES, S., 1925, *J. Bact.*, **10**, 569.
 BERGEY, D. H., 1923, *Bergey's Manual of Determinative Bacteriology*, Baltimore
 BIRT, C. & LEISHMAN, W. B., 1902, *J. Hyg., Camb.*, **2**, 120.
 BOLLINGER, O., 1877, *Zbl. med. Wiss.*, **15**, 481.
 BOSTROEM, 1891, *Beitr. path. Anat.*, **9**, 1-240, 10 pl.
 BRUMPT, E., 1906, *Arch. Parasit.*, Paris, **10**, 489.
 BUCHANAN, R. E., 1925, *General Systematic Bacteriology*, Baltimore.
 CHALMERS, A. J. & ARCHIBALD, R. G., 1916, *Ann. Trop. Med. Parasit.*, **10**, 169.
 CHALMERS, A. J. & CHRISTOPHERSON, J. B., 1916, *Ann. Trop. Med. Parasit.*, **10**, 223.
 CHRISTOPHERSON, J. B. & ARCHIBALD, R. G., 1918, *Lancet*, ii, 847.
 COLEBROOK, L., 1920, *Brit. J. Exp. Path.*, **1**, 197; 1921, *Lancet*, Lond., i, 893.

- COPE, V. Z., 1915, *Brit. J. Surg.*, **3**, 55.
- CORNWALL, J. W. & LAFRENAIS, H. M., 1922, *Ind. J. Med. Res.*, **10**, 239.
- DAVAINE, 1850, *C.R. Soc. Biol.*, Paris, 1851, **2**, 119.
- DRESCHLER, 1919, *Bot. Gaz.*, **67**, 147.
- EPPINGER, H., 1891, *Beitr. path. Anat.*, **9**, 287, cited from *Zbl. Bakt.*, 1891, **9**, 274.
- DA FONSECA, O. & LEÃO, A. E. DE A., 1927, *Bol. Inst. Brasileiro Sci.* (Rio de Janeiro), **3**, 24.
- FORD, W. W., 1927, *Text-Book of Bacteriology*, Philadelphia and London (Saunders).
- FÜLLEBORN, F., 1911, *Arch. Schiffs- u. Tropenhyg.*, **15**, 131.
- GILBERT, 1904, *Z. Hyg. InfektKr.*, **47**, 383.
- GORDON, M. H., 1920, *Brit. Med. J.*, i, 435.
- GRIFFITH, F., 1916, *J. Hyg.*, Camb, **15**, 195.
- GUNST, J. A., 1927, *Tijdschr. Diergeneesk.*, Deel 54.
- HARBITZ, F. & GRÖNDAHL, N. B., 1911, *Beitr. path. Anat.*, **50**, 193.
- HARZ, C. O., 1877-8, *Jahresb. Centr.-Thierarznei-Schule in München*, Leipzig, **1879**, 125.
- HENRICI, A. T. & GARDNER, E. L., 1921, *J. Infect. Dis.*, **28**, 232.
- HIGGINS, 1908, *Proc. Amer. Vet. Med. Ass.*
- HÜLPHERS, 1923, *Svensk. VetTidskr.*, s 273.
- ILLICH, A., 1892, *Beitrag zur Klinik der Aktinomykose*, Wien, pp. 201 (full bibliog.).
- ISRAËL, J., 1887, *Arch. klin. Chir.*, **34**, 160.
- ISRAËL, JAMES, 1878, *Arch. path. Anat.*, **74**, 15-43, 4 pl.; 1879, *ibid.*, **78**, 421.
- ISRAËL, OSCAR, 1884, *Arch. path. Anat.*, **95**, 140.
- JUDD, W. R., 1926, *Brit. Med. J.*, ii, 886.
- KANTHACK, A. A., 1893, *J. Path. Bact.*, **1**, 140.
- KLINGER, R., 1912, *Zbl. Bakt.*, Abt. 1, Orig., **62**, 191.
- LANGERON, M., 1922, *Nouveau Traité de Médecine* by G. H. Roger, Fernand Vidal and P. J. Teissier, Fasc. IV, p. 404.
- LAVERAN, 1902, *Bull. Acad. Méd.*, Paris, **47**, 773; 1906, *C.R. Soc. Biol.*, Paris, **61**, 340.
- LEBERT, H., 1857, *Traité d'anat. path. gén. et. speciale*, Tome I, 54, Plate II, fig. 16.
- LEHMANN, K. B. & NEUMANN, R. O., 1927, *Bakteriologie*, München.
- LIESKE, R., 1921, *Morphologie und Biologie der Strahlenpilze (Actinomyces)*, Leipzig.
- LIGNIÈRES, J. & SPITZ, G., 1902¹, *Bull. Soc. cent. Méd. vét.*, n.s., **20**, 487, 546; 1902², *Rev. Soc. méd. argent.*, N. 53; 1904, *Zbl. Bakt.*, Abt. 1, Orig., **35**, 294, 452.
- LORD, F. T., 1910, *J. Amer. Med. Ass.*, **55**, 1261.
- MCINTOSH, J. & FILDES, P., 1916, *Lancet*, Lond., i, 768.
- MAGNUSSON, H., 1922, *Svensk. VetTidskr.*, **27**, 121; 1928, *Acta path. microb. scand*, **5**, 170.
- MASON, F. E., 1919, *J. Comp. Path.*, **32**, 34.
- MUSGRAVE, W. E. & CLEGG, M. T., 1907, *Philipp. J. Sci.*, **2**, 477.
- NAESLUND, C., 1925, *Acta path. microb. scand.*, **2**, 110; 1926, *ibid.*, **3**, 637; 1929, *ibid.*, **6**, 66.
- NATIONAL COLLECTION OF TYPE CULTURES, 1925, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 64 (revised).
- NICOLLE, C. & PINOY, E., 1908, *Bull. Soc. Path. exotique*, **1**, 95.
- NOCARD, E., 1888, *Ann. Inst. Pasteur*, **2**, 293; 1902, *Bull. Soc. cent. Méd. vét.*, p. 695.
- PERRONCITO, E., 1879, *Deuts. Z. Thiermed.*, **5**, 33, 1 pl.
- PIJPER, A. & PULLINGER, B. D., 1927, *J. Trop. Med.*, **30**, 153.
- PINOY, E., 1913, *Bull. Inst. Pasteur*, **11**, 929 and 977.
- PONFICK, 1879, *Berl. klin. Wschr.*, **16**, 345; 1882, *Die Actinomykose, eine neue Infektionskrankheit, etc.*, Berl., pp. 132, 6 pl.
- RIVOLTA, S., 1882, *Arch. path. Anat.*, **88**, 389.
- SCHUKIEWITSCH, J. J., 1903, cited from *Jber. Leist. ges. Med.*, **37**, 711.
- SCHÜTZE, H., 1908, *Arch. Hyg. Berl.*, **67**, 35.
- SEMON, H. C., 1915, *Proc. R. Soc. Med.*, **8**, Dermat. Sect., 172.
- SHIOTA, H., 1909, *Deuts. Z. Chir.*, **101**, 291.
- SILBERSCHMIDT, 1899, *Ann. Inst. Pasteur*, **13**, 841.
- SLESINGER, E. G., 1920, *Lancet*, Lond., i, 1220.
- THIROUX, A. & PELLETIER, J., 1912, *Bull. Soc. Path. exotique*, **5**, 585.

- TOPLEY, W. W. C. & WILSON, G. S., 1929, *The Principles of Bacteriology and Immunity*, 2 vols., London.
- VINCENT, H., 1894, *Ann. Inst. Pasteur*, **8**, 129.
- WAKSMAN, S. A., 1927, *Principles of Soil Microbiology*, London.
- WELCHMAN, W. & PIRIE, J. H. HARVEY, 1921, *Med. J. S. Afr.*, **17**, 6.
- WOLFF, M. & ISRAËL, J., 1891, *Arch. path. Anat.*, **126**, 11.
- WRIGHT, A. E., 1924, *Lancet*, Lond., i, 218.
- WRIGHT, J. H., 1904-5, *J. Med. Res.*, **13**, 349.

CHAPTER III. PATHOGENIC LEPTOTHRICEÆ.

BY IVY MACKENZIE (GLASGOW).

General Description and Classification.

THE Leptothriceæ belong to the higher bacteria. The term Leptothriceæ was first used by Kuetzing (1843) for a family group of slender filamentous organisms found in iron springs and in marshy ground. He proposed the generic designation *Leptothrix* to include the four species described ; and one of these, *L. ochracea*, is used by Ellis (1907) as the type in his description of the genus. *L. buccalis*, which has received some notice in medical literature, was first described by Robin (1847), who found it on the teeth of a horse. It has been taken by Heim (1911) as the type of the genus, and he says it is found in the mouth in the form of unbranched thread-like structures which stain yellow with iodine. He remarks that there are, also, in disease of the mouth, filamentous organisms which appear in granular conformation, stain violet with iodine, and resemble in appearance the actinomycotic granule. He adds that more precise description cannot be given because culture is impossible. Kligler (1915), however, in a study of the organisms of the teeth, succeeded in cultivating fifteen strains of *Leptothrix buccalis*, which he describes as a thick, long, straight or curved thread with a club end at one extremity and a tapering end at the other. As a rule 0.8μ thick and 10μ and upwards in length, it stains homogeneously in young cultures, while in older cultures it is represented by a faintly stained sheath enclosing heavily stained granules. Treated by Gram's method the young cultures retain the stain ; in old cultures the sheath is decolorized while the granules are deeply stained. They do not grow on agar, but on glucose-agar plates appear as pin-point colonies in 3 to 4 days ; they grow in serum-glucose-agar, best in stab culture, with good growth in the depth and slight on the surface ; the addition of salivary mucinate to agar improves the medium ; glucose, sucrose and maltose are fermented, lactose is not affected ; gelatin is not liquefied and neither indole nor ammonia produced. The optimum temperature is 37°C . and there is practically no growth at 20°C .

According to Lehmann and Neumann (1927) the leptothrix group and the family to which it belongs (Desmobacteriaceæ) is more closely allied to the lower than to the higher bacteria, and the diphtheria and tubercle bacilli occupy an intermediate position in phylogeny between the Leptothriceæ and the Actinomycetaceæ which, admittedly, belong to the Hyphomycetes. It should be noted, however, that in their description of the Desmobacteriaceæ they suggest that the species belonging to this family show a closer relationship with the algæ than any of the other lower bacteria. They point out that, although there is no true branching,

it is possible in many species to distinguish in the filament a basal end, which is fixed, from a freely growing apical end. The ends may vary in thickness and there are no endospores.

They include in the family, as do practically all earlier systematists, the following groups: (1) *Beggiatoa*, (2) *Leptothrix*, (3) *Crenothrix*, (4) *Cladothrix*, and (5) *Thiothrix*. There is a further division of *Leptothrix* into (a) *Leptothrix (sensu strictiori)*, about which it is remarked that it is very difficult to cultivate and requires further investigation, and (b) *Chlamydothrix (Migula)*, which includes *L. ochracea* and the iron bacteria. The most significant feature of the description of *Desmobacteriaceæ* by Lehmann and Neumann is the absence of any suggestion that any species of the family group may be credited with pathogenic activity in the human subject.

In their systematic classification of bacteria (1925) the Committee of American Bacteriologists assign to the *Leptothricia* a place which implies a closer relationship to the higher orders of bacterial life. In the final report of 1920 the Committee recognizes an order, *Actinomycetales*, which is divided into two families: (1) *Actinomycetaceæ*, and (2) *Myobacteriaceæ*. The artificial key to the family *Actinomycetaceæ* indicates the relative position of the *Leptothricia* in this arrangement:

- A—Typically filamentous forms *Actinomycetaceæ*.
- B—Mycelium and conidia formed *Actinomyces*.
- BB—No true mycelium.
- C—Cells show branching.
- D—Gram-negative *Actinobacillus*.
- DD—Gram-positive *Erysipelothrix*.
- CC—Cells never branch Gram-positive
threads later fragmenting into rods .. *Leptothricia*.

Although the *Leptothriceæ* have a widespread and varied distribution in nature, the attempts to give precise definition to their morphological and biological characters have not, so far, been successful. Botanists have been interested, for the most part, in those forms which infest water, and especially water which contains iron; the great mass of bacteriological literature is concerned with the species which inhabit the mouth and adjacent ducts and cavities; clinical and pathological observations are related, as a rule, to diseases of the mouth and pharynx and to sporadic and unusual cases of meningitis, many or most of which are probably the result of infection by a variant of the bacillus of influenza. The difficulty in determining the position and assessing the pathological importance of the *Leptothriceæ* arises from the fact that they do not lend themselves readily to the methods of artificial cultivation, and thus lack the properties which provide the criteria for systematic discrimination. A review of the literature on the subject reveals so much discrepancy in respect of staining reactions, motility and spore formation, that one is entitled to regard the whole subject as being still in a state of chaos. In all probability the *Leptothrix* tends to extreme variation.

Pathogenicity.

There are no well-defined disease processes comparable, for example, with actinomycosis, which can be attributed with certainty to leptothrix infection. Huntemüller (1927) attributes the infrequency of infection to the absence of favourable conditions of growth. There are, however, in medical literature numerous records of cases in which filamentous organisms in the seats of reaction have been regarded as the causal agents. The majority of these cases comprise a mouth disease, known as keratosis pharyngis, and also referred to as mycosis pharyngis, leptothricia, pharyngomycosis, mycosis tonsillaris benigna, &c. Brown Kelly (1896) has given a summary of the literature, as well as an account of his own observations of this disease, in his *Mycosis Pharyngis Leptothricia and Keratosis Pharyngis*. He distinguishes between the two conditions mentioned in the title: keratosis pharyngis he regards as primarily a patchy cornification or keratosis of the epithelium of the oropharynx, which incidentally provides for the proliferation of a saprophytic leptothrix; the patients appear, as a rule, to be in good health: on the other hand, in mycosis pharyngis leptothricia he recognizes a true mycosis in which there is inflammation of the pharynx with white patchy exudation and painful deglutition, and the bulk of the exudate contains masses of leptothrix which are supposed to be '*Leptothrix buccalis*'. So far, however, bacteriological technique has failed to provide evidence of the differential features of the organism or of its pathogenic relation to the disease. The problem of the biological characters of the leptothriceæ of the oral and nasopharyngeal cavities and their relation to disease in these parts has yet to be solved.

Among the earlier records of leptothrix infection are those describing inflammation of the tear-ducts, in which the organism was present in abundance. In 1855 A. von Graefe published a case in which small nodules of massed filaments were noted in the inflamed eye. In 1869 he published an account of ten cases with concretions and filamentous organisms in the tear-ducts. The filaments differed from those of *Leptothrix buccalis* in that they did not give an iodine reaction. Detailed accounts of cases have been given by Hirschberg (1902), Segelken (1902), Wirtz (1922) and Wanka (1923). It is significant that it was not possible to obtain growth on artificial media from any of those cases, although the film preparations indicated that the leptothrix was present in abundance and in pure culture. The presence of concretions in the affected tear-ducts is of interest in connection with the suggestion that the keratosis of the epithelium of the pharynx is related to the leptothrix infection of that region. It is suggested that the tear-duct infection arises from organisms originally present in the mouth.

Film preparations in some cases of putrid and gangrenous bronchitis show large numbers of filamentous organisms which, presumably, come from the mouth; in these pulmonary conditions there is such a variety of organisms that it is impossible to assess the pathogenic importance

of the leptothrix. Matthews (1905), however, described a case of localized empyema in which the only organism found was a Gram-negative leptothrix. Attempts to procure artificial cultivation were not successful, but the organism was regarded as *Leptothrix racemosa vincenti*.

The presence of filamentous organisms in gastric ulcers described by Löhlein (1920) cannot be regarded as evidence of their pathogenic activity; but an interesting case of duodenal ulcer associated with infarctions of the liver containing abundant masses of Gram-negative filamentous and coccal forms is recorded by F. J. Lang (1920). Grey-green nodules, varying in size from a hemp-seed to a hazel-nut, shone through the liver capsule. On microscopic examination the nodules were found to be related to the bile-ducts, whose walls were thickened with connective tissue and whose lumina were widened and filled in parts with denuded and necrotic epithelium. There were abundant non-branching filaments and masses of cocci, both Gram-negative. The surrounding portal veins and capillaries contained similar organisms and the adjacent liver cells were necrotic. There was a notable absence of purulent exudate. Cultures were not made at the post-mortem examination, but the case is important in that it suggests a leptothrix infection, and the presence of Gram-negative coccal elements among the Gram-negative filaments corresponds to the findings in cases of meningitis to which reference will be made later.

The only record of an epidemic spread of disease attributed to leptothrix infection is recorded by Blumer and McFarlane (1901), who give an account of sixteen cases of noma which occurred in the convalescent stage of measles. The noma affected different parts of the body, involving the mouth alone in four cases, while the mouth, ear, vulva, rectum and other parts were affected in varying combinations in the other cases. Bacteriological examination, which was carried out in the majority of the cases, showed the presence of a leptothrix organism, apparently in pure culture in the early stages of the disease, but mixed with coliform and streptococcal organisms later. The filaments were 0.5μ broad and 5 to 20μ long, were unbranched, Gram-positive and easily stained. Cultural experiments were not made.

Leptothrix Meningitis.

In the general literature of medicine there are accounts of meningitis due to filamentous organisms resembling leptothriceæ. D'Este Emery (1904) published an account of three cases of meningitis in which he had grown, from the cerebrospinal fluid, a Gram-negative bacillus not previously described; it was pleomorphic, diplococcal and filamentous. It grew readily in nutrient agar; there was no further bacteriological test. Darling and Wilson (1907) described in a case of meningitis a pleomorphic organism with coccal and bacillary forms of varying size. Stuart M'Donald (1908), in a communication dealing with observations on epidemic cerebrospinal meningitis, refers to 'the occurrence of leptothrix forms in the cerebrospinal fluid in cases of acute meningitis'. The

films in one case showed diplococcal forms and long, thin, irregularly and faintly stained bacilli. They were Gram-negative and there was no growth on blood- or serum-agar. In a second case in which the films showed elongated diplococci and long bacillary forms, also Gram-negative, growth took place on blood-agar in three days. On the third day, colonies resembling those of pneumococci appeared and films showed long, thin bacilli which, on the fourth day, appeared as masses of sinuous filaments; they were Gram-negative.

Stoevesandt (1908) published a paper dealing with organisms other than meningococci isolated by him from cerebrospinal fluid in the 1906 epidemic in Kiel. He refers to pleomorphic organisms which appear as diplococci, short rods and long filaments stretching across the field of the microscope. Cohen (1909) described a Gram-negative bacillus isolated from three cases of septicæmic cerebrospinal meningitis. The organism was present in the cerebrospinal fluid in all three and was isolated from the blood in two; all cases ended fatally. There was growth on blood-agar only and in dew-drop colonies. The organism was cocco-bacillary and filamentous, non-motile, aerobic and pathogenic to rabbits.

Ritchie (1910) described three cases of 'Meningitis associated with an Influenza-like Bacillus'. The organism was Gram-negative, grew on blood-smeared agar only, and was non-motile. In breadth it was 0.3 to 0.4 μ and in length it varied up to 10 or 15 μ . In the earlier growths it tended to vary considerably in shape and size, but after some months' reinoculation in blood-agar the bacilli were small and did not exhibit the variation seen in the earlier stages. Stuart M'Donald and Slade (1912) made a further communication 'On Leptothrix Meningitis with an account of two cases associated with a Hæmophylic Bacillus'. The films from the cerebrospinal fluid showed Gram-negative bacilli of varying length and breadth and the only original growth was on human blood-agar. After daily culture on blood-agar for a week there was growth on nutrient-agar, gelatin and in broth. Litmus became acid in 24 hours and, again, alkaline between the eleventh and twenty-first days. The indole test was negative. The sugar reactions showed, in 96 hours, acidity in saccharose, maltose, glucose, dextrose, salicin and mannitol, and no acidity in lactose, raffinose, inulin, sorbitol and dulcitol. While the earlier growths showed very considerable variation in the length of the organism, the bacilli, after a few weeks' recultivation, assumed a more constant shape and length, remaining short with an occasional long filament.

These publications are representative of a large series in which Gram-negative bacilli have been described in meningitis. In German literature they are referred to as 'typhus-ähnliche' bacilli. In English, American, French and Italian literature their resemblance to *B. influenzae* is emphasized.

It is, of course, well known that the influenza bacillus tends to variation and may appear in mass filaments under certain conditions. Filamentous organisms in the coli-typhoid group are also well known, and variants of

these may, in some instances, show a tendency to mass-filament forms under certain cultural conditions. All these communications, however, afford no conclusive evidence that the filamentous organism belongs to the higher group of bacteria. The descriptions given resemble in some respects, however, the findings in cases of meningitis observed by the writer, where experimental cultivation of the organism showed changes in growth and behaviour justifying its designation as a true leptothrix.

The Writer's Observations.

From a series of 25 cases in which Gram-negative organisms other than meningococci were present in the cerebrospinal fluid, it was possible in 13 to obtain cultures on suitable media, and in 6 of those 13 cases the organisms, on artificial cultivation, showed features which suggested a relationship with the higher bacteria. The special feature was their capacity for appearing in two phases: (1) a pre-sporing or non-sporing phase in which they resemble lower bacteria and are killed at 60° C. in half an hour, and (2) a sporing phase in which spores are formed at 22° C. and after resisting a temperature of 80° C. for half an hour grow into mass filaments in 18 to 48 hours at 37° C., and in the filamentous state they are killed at 60° C. in half an hour.

Of the 6 cases referred to, 2 died, 4 made a perfect recovery, and in 1 of those 4 a recurrence of the infection took place after an interval of six months, from which the patient again recovered. The cultures from the cerebrospinal fluid grew on blood-agar only at the commencement, but after recultivation were easily grown on ordinary agar. In 2 of the cases the organism was recovered from the blood in glucose broth cultures. In 2 of the cases the organism was found in the sporing stage only, and in the other 4 cases the organism passed from a pre-sporing to a sporing phase under artificial cultivation, so that in 4 of the cases both a sporing and pre-sporing strain were kept for experiment.

The organisms appear all to belong to one species. The pre-sporing strains respond in the same way to the standard biological tests, and the sporing strains, though differing slightly from the pre-sporing in respect of chemical reaction and greatly in respect of environmental response and morphological conformation, correspond with each other in every detail.

Pre-sporing strains.

Film preparations from the cerebrospinal fluid showed small clusters of Gram-negative bacilli with occasional filaments and diplococcal forms. Cultures on blood-agar gave in three days minute greyish, translucent colonies. Early colonies showed bacillary and cocco-bacillary forms with few filaments, all Gram-negative. After 48 hours the filaments were 10 to 12 μ long and 0.5 μ broad, and in another two days mass filaments were formed. There was no growth on gelatin or potato. Growth was aerobic only. After some months the organisms tended to become more uniform in shape and filaments fewer. After some months growth occurred at 22° C., and after four years the growth was good both

at 22° C. and at 37° C. After four years, pleomorphism in ordinary media had completely disappeared, though staining reactions remained the same.

Motility. (1) Active motility was seen in broth after 18 hours at 37° C.; (2) after 3 days on agar at 37° C. and then 3 days at 22° C. there was very little motility.

Chemical reactions. Sugars were not fermented. Milk was made alkaline. Indole was not produced. In 3 per cent. potassium iodide in broth, long filaments with bulbous formations were obtained.

Growth from pre-sporing to sporing strains.

In two of the cases the sporing phase had already occurred in the original cultures from the cerebrospinal fluid. In one case (the case in which there were two illnesses) the sporing phase occurred spontaneously at 22° C. about two months after the original isolation of the organism. In three other cases sporing occurred only after growth on glucose-agar to which 1 per cent. disodium-glycerophosphate had been added; here, again, sporing occurred at 22° C. only.

Sporing strains.

In the cases from which the sporing strains were isolated direct from the cerebrospinal fluid the film preparations from the fluid showed large filamentous forms, in addition to bacillary, cocco-bacillary and diplococcal organisms. They were all Gram-negative.

Cultural growth. There was good growth on agar at 37° C. The colonies were small and opaque with translucent edges. There was no alkalinity in milk and no indole reaction. Growth at 22° C. in 24 hours resembled incubated cultures. Growth occurred under aerobic conditions only. The organism may continue to live in the sporing phase for three years at least.

Microscopic appearance and staining reactions varied according to the age of the culture and according as growth occurred at 37° C. or 22° C. After 18 hours on agar at 37° C. there were mass filaments 10 to 50 μ long, unbranched and partly decolorized by Gram. In three or four days the filaments broke down by septal division into shorter segments and bacilli. There were clusters of small bacillary and cocco-bacillary organisms among the larger filaments. Cultures grown at 37° C. were always killed in half an hour at 60° C. A filamentous growth (at 37° C.) left at 22° C. in 18 hours showed dividing of the filaments into bacilli, which became pointed at the ends and thickened at the centre, and took on a special stain for spores (fuchsine and methylene blue after rapid decolorization). Subculture at this stage left again at 22° C. reproduced the same type of colony, consisting of only Gram-negative bacilli, some of which after a week showed spore formation. Spores appeared only in isolated bacilli, and in a few clusters, in contrast with their general formation in *B. subtilis*, for example. Cultures grown for 10 days at 22° C. always resisted 80° C. for half an hour.

Motility was present in a certain phase of development of the sporing strain. After two days' growth at 37° C. organisms were filamentous and non-motile. If filaments be placed on agar-covered slides at 22° C. they show at first slow wavy movement, and afterwards become more active, and as they break down into bacilli, the organisms move about with great rapidity. A growth on agar at 22° C. for 3 days is very motile. If, however, a culture be grown on agar at 37° C. for 6 days and then at 22° C. for 10 days till spores are produced, there is no motility, but a subculture from this on fresh agar at 22° C. shows motility in a day or two.

Chemical reactions. Sugars were not fermented. Milk was not made alkaline, as it was by the pre-sporing strains. Indole was not produced. In 5 per cent. potassium iodide mass filaments with bulbous thickenings were produced, comparable with those obtained by the action of 3 per cent. potassium iodide on pre-sporing strains.

Conversion of non-sporing into sporing phase.

After a time the pre-sporing phase became stabilized on agar and it was impossible to induce sporulation with disodium-glycerophosphate. The pre-sporing strains were four years old when the following procedure was tried. An agar growth was inoculated in 4 c.cm. of 30 per cent. saccharose in broth at 37° C. In 12 to 15 days this was plated out on agar and kept at 37° C. for 3 days and then left for 12 days at 22° C. By this time a creeping, translucent fringe surrounded the individual colonies, and from this fringe a second inoculation was placed in saccharose and the whole experiment repeated. A growth from the dewy fringe after the second experiment was found to resist 80° C. for half an hour, and the resistant growth appeared in two days at 37° C. in the form of long filaments, Gram-negative, non-motile, and killed at 60° C. in half an hour. The non-motile filaments inoculated on agar and placed at 22° C. broke down into bacilli and became actively motile; and when left for 10 days at 22° C. they showed isolated spores retaining the special stain and resisting heat at 80° C. for half an hour. A subculture of these for two days at 37° C. once more gave rise to filaments which were killed in half an hour at 60° C.

Conversion of sporing into non-sporing phase.

It was possible by pursuing a similar method to isolate daughter-colonies from the sporing phase, which, when subcultured, failed to produce spores.

Conclusions.

The occurrence of filamentous forms is not of itself evidence of relationship with the leptothriceæ; it is known in the bacilli of typhoid, dysentery and influenza, and more especially in early cultures of *B. coli* from the urinary tract: but in the cases of meningitis described the pleomorphism found expression in forms and in transformations unknown in these bacteria. The bi-phasic potentiality of the organism, as revealed in sporing and non-sporing strains with their mutual convertibility, is not more

significant than the peculiar behaviour of the sporing strain itself. The sporing strain grown at 22° C. is uniformly Gram-negative and produces no filaments; at 37° C. it is filamentous and varies in its reaction to Gram, some filaments being positive, others negative, and others partly positive and partly negative; the filaments are non-motile so long as they remain at 37° C., but placed at 22° C. they show wavy, sinuous movements, and the bacilli into which they divide at this temperature become actively motile; in older cultures which have spored this motility has disappeared. When, in addition to these phenomena, it is noted that (1) the spores themselves or the conditions of their development differ from those of the lower bacteria, and that (2) in the earlier observations there were among the mass filaments clusters of smaller organisms (diplococci and diplobacilli) which could not be separated by plating, there is an accumulation of peculiar features which proclaims an advance on the fission fungi.

The organism seems to occupy a place on a phylogenetic plane with the actinomyces. More complicated than the simple bacteria, and apparently more complicated than the bacilli of diphtheria or of tubercle, the sporing strain itself, when first isolated, presented microscopically all the pleomorphic characters described by Eppinger (1891) in his '*Cladothrix asteroides*'. In Eppinger's organism the filaments were thought originally to show false branching, although now the branching is regarded as true (streptothrix); in respect of the variety and shape of the smaller concomitant microbes, coccoid, bacillary and spiral, the description corresponds, in practically every detail, with what was seen in the filamentous phases of the sporing strains when they were first isolated.

It is feasible to suggest that the relation of the non-sporing to the sporing strain resembles that of the *Bacillus actinomyces* *comitans* with the mycelium of actinomyces (see also p. 85). This small (0.5 to 1 μ) non-motile, Gram-negative coccoid or cocco-bacillary organism associated with the mycelial growth in actinomycotic lesions, may be isolated by plating, but it is difficult to obtain a growth of the mycelium free from the concomitant. Klinger (1912), who first described it, looked upon it as a distinct organism, while Colebrook (1920), who has studied it in a large series of cases, has suggested a possible genetic relationship with the mycelium. Mackie (1927), in his observations on two strains of actinomyces, found it impossible, after repeated plating and subculturing single isolated colonies, to separate the mycelium from the concomitant. A growth of the latter developed invariably from the mycelium organism. After two years' culture, however, one of two strains showed mycelium only. These phenomena are, perhaps, not identical with what has been described in the case of the non-sporing and sporing strains, but they are so similar as to justify the inclusion of these strains in the higher bacteria. In view of the non-branching character of the filaments, they must be regarded as belonging to the leptothriceæ, and the following designation for the species is proposed—*Leptothrix hæmoglobinophila sporulans*.

It might be suggested that the artificial nature of the means employed to elicit the peculiar characters of this organism would be capable of provoking similar reactions in other species, which, judged by the ordinary standards of reference, belong to the lower orders of microbic life. Of this, however, there is, so far, no evidence; for, although the recent and highly important researches of Arkwright, Mellon, Hadley and others, on 'dissociative reaction' and 'variation' give promise of a fuller understanding of the biological changes which underlie 'infectivity' and 'virulence', they do not imply any confusion or misleading simplification in the taxonomic arrangement, such as it is, whereby the 'lower' are placed in a class apart from the 'higher' bacteria.

REFERENCES.

- BLUMER & MCFARLANE, 1901, *Amer. J. Med. Sci.*, **122**, 527.
 BROWN KELLY, A., 1896, *Mycosis Pharyngis Leptothricia and Keratosis Pharyngis*, Glasgow.
 BUCHANAN, R. E., 1925, *General Systematic Bacteriology*, Baltimore.
 COHEN, 1909, *Ann. Inst. Pasteur*, **23**, 273.
 COLEBROOK, L., 1920, *Brit. J. Exp. Path.*, **1**, 197.
 DARLING, J. S. & WILSON, W. J., 1907, *Brit. Med. J.*, **i**, 433.
 ELLIS, D., 1907, *Zbl. Bakt.*, Abt. II, **19**, 516.
 EMERY, W. D'ESTE, 1904, *Lancet*, Lond., **ii**, 593.
 EPPINGER, H., 1891, *Beitrag. path. Anat.*, **9**, 287.
 V. GRAEFE, A., 1869, *Graefes Arch.*, **15**, 318.
 HEIM, L., 1911, *Lehrbuch der Bakteriologie*.
 HIRSCHBERG, 1902, *Zbl. Augenhk.*, **26**, 7.
 HUNTEMÜLLER, O., 1927, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **5**, 2.
 KLIGLER, I. J., 1915, *A Biochemical study and differentiation of Oral Bacteria, with special reference to Dental Caries*, Diss. Ph.D., Columbia University.
 KLINGER, R., 1912, *Zbl. Bakt.*, Abt. I, Orig., **62**, 191.
 KUETZING, F. T., 1843, *Phycologia Generalis*.
 LANG, F. J., 1920, *Virchows Arch.*, **234**, 367.
 LEHMANN & NEUMANN, 1927, *Bakteriologische Diagnostik*, 7 Auflage.
 LÖHLEIN, M., 1920, *Virchows Arch.*, **227**, 86.
 M'DONALD, S., 1908, *J. Path. Bact.*, **12**, 446.
 M'DONALD, S. & SLADE, 1912, *N. Engl. Clin. J.*, April.
 MACKIE, T., 1927, cited from Muir and Ritchie, *Manual of Bacteriology*, 8th edition, p. 375.
 MATTHEWS, P., 1905, *Pract.*, **74**, 197.
 RITCHIE, J., 1910, *J. Path. Bact.*, **14**, 615.
 ROBIN, CH., 1847, *Des Végétaux qui croissent sur les animaux vivants*, Paris.
 SEGELKEN, 1902, *Klin. Mbl. Augenhk.*, **40**, 2, 134.
 STOEVEsandt, K., 1908, *Zbl. Bakt.*, Abt. I, Orig., **46**, 295.
 WANKA, 1923, *Ber. Deuts. ophth. Ges. Tschechoslowakei*, cited from *Klin. Mbl. Augenhk.*, **71**, 237.
 WIRTZ, 1922, *Klin. Mbl. Augenhk.*, **68**, 385.

CHAPTER IV. THE SPIROCHÆTES.

BY EDWARD HINDLE,
THE BEIT RESEARCH FELLOW IN TROPICAL MEDICINE
(WELLCOME BUREAU OF SCIENTIFIC RESEARCH, LONDON).

Introduction.

THE micro-organisms constituting the large and important group known as spirochætes are almost as ubiquitous as ordinary bacteria, yet owing to a combination of circumstances, their study has been somewhat neglected. One of the reasons for this neglect is the uncertainty as to whether they should be regarded as animals or plants; bacteriologists in general have left them to be dealt with by protozoologists, and the latter, for the most part, have little interest in organisms of such doubtful animal affinities.

Spirochætes cannot be identified by using the ordinary morphological methods of the protozoologists, yet with few exceptions their cultural requirements more closely resemble those used for parasitic protozoa than for ordinary bacteria. It is, therefore, not surprising that our knowledge of the group is still very incomplete, although the creation of a bewildering number of names, and numerous serological comparisons of various strains, might give a contrary impression.

Leeuwenhoek, in 1681, was the first to discover these organisms, and he recorded their presence in the human mouth and intestine (see Dobell, 1923), but it was not until 150 years later that the earliest named spirochæte, *S. plicatilis*, a large free-living form, was described by Ehrenberg (1834). The group then received comparatively little attention until the present century, although the discovery by Obermeier (1873) in Russia of spiral organisms, *S. recurrentis*, in the blood of relapsing-fever patients aroused some interest at the time. Within the last 30 years, however, many pathogenic forms have been discovered, such as the causative organisms of syphilis, yaws, spirochætal jaundice, relapsing fevers in man and animals, &c., and also spirochætes associated with conditions such as Vincent's angina, bronchial spirochætosis, and various ulcerations. In addition to forms which are definitely pathogenic, there are spirochætes inhabiting the mouth, alimentary tract and genitalia of many vertebrates, and nearly all mammals, which seem to be harmless saprophytes, although in some instances these supposedly saprophytic forms are found associated with diseased conditions and may have some pathogenic significance.

Spirochætes are also found in the bodies of invertebrates; all the members of one genus, *Cristispira*, live in the crystalline styles of lamellibranchs. Finally, there are very many free-living forms in the sea, rivers, ponds, &c., and also in damp soil.

Generally the presence of organic matter in water favours the growth of spirochætes, and consequently they are more abundant in cesspools,

drains and similar localities, especially if the water contains sulphuretted hydrogen. So far as is known, all spirochætes are unable to withstand drying, and consequently they cannot be spread by currents of air, as can many bacteria.

General Description.

Definition.

The spirochætes constitute a fairly distinct group of organisms, characterized by their spiral form, flexibility, and the power of active movement without the aid of flagella or any other organs of locomotion, except possibly in the case of developmental forms which may show a spiral terminal flagellum. They are most closely related to the non-flexible *Spirilla*, and in fact many spirochætes, such as *S. recurrentis*, are still erroneously called by this name in some of the current text-books.

The group has been the subject of many voluminous and also acrimonious discussions as to their nature, affinities, structure, life-history and nomenclature, and no general agreement has been reached on many of these questions. It will easily be understood that organisms such as these, many of which approach (if they do not pass) the limit of vision, even with the aid of the latest microscopical appliances, are particularly liable to subjective interpretations, and anyone who has had any experience of the pathogenic forms will realize some of the difficulties attending their observation.

With regard to the smaller forms it is not possible to determine any definite internal structure, and therefore if we assume, as seems probable, that the spirochætes are a homogeneous group, greater weight should be attached to the study of the larger species, such as *Spirochæta plicatilis*, which are not so difficult of observation as the pathogenic forms.

*Form. (Figs. 1 to 25.)**

The ordinary spirochætes belonging to the genus *Spirochæta* have the form of a simple right-handed spiral with tapering or rounded extremities, but in the subgenus *Leptospira* the spiral is very closely wound and sometimes a second spiral appears to be superimposed on it. The larger species, such as *S. plicatilis* (Figs. 1 & 2), contain a distinct axial rod or filament, sometimes giving the appearance of an undulating membrane. It is very difficult to decide whether this axial filament is present in the smaller forms, but since the larger forms clearly show such a structure, and there is some evidence for its existence in the smaller forms (see Noguchi, 1928), the author agrees with Zuelzer (1925) that its presence may be assumed, since there is close resemblance in every other particular. On this assumption there is no very clear morphological distinction between the large water spirochætes and the pathogenic forms.

The body of the spirochæte is covered with a limiting membrane, and as a rule distinct organs of locomotion, such as cilia or flagella, are absent.

* The figures referred to in this and subsequent chapters on spirochætes are arranged on two plates at the end of this chapter.

The terminal flagella that have been described, especially in *Spirochæta pallida*, may be the result of the shrinkage of the cytoplasm, leaving the empty limiting membrane, or possibly the axial filament, of the spirochæte protruding from one or both of the extremities. Such structures may be observed after division when the ends of the daughter-individuals are drawn out in the form of fine filaments, but in short forms a true terminal flagellum may be present.

The so-called lateral flagella, obtained after certain methods of fixation and staining, seem to be artefacts produced by the body of the spirochæte bursting and liberating its contents in filamentous form.

In the larger species belonging to the genus *Cristispira* (Figs. 3-6), the body has a chambered structure and a form of chromidial nucleus may be present. Metachromatic granules (volutin) have been demonstrated in *S. plicatilis*, and according to Zuelzer (1925) are also present in some of the other species, such as *S. eurystrepta*.

The method of multiplication is always by transverse division, either simple or multiple, consequently under similar conditions the thickness of any individual remains fairly constant, although considerable variations in length occur, as a result of growth after division. When spirochætes are grown in different culture media they seem to show variations in thickness, and similar changes have been recorded in blood spirochætes after being ingested by the tick or insect that serves as the transmitting host. When dividing, the two halves sometimes become entwined preparatory to final separation, and these forms may very easily be mistaken for examples of longitudinal division (Fig. 6).

Movement.

Antero-posterior polarity is completely absent in all spirochætes (Dobell, 1912²) and consequently these organisms move indiscriminately in the direction of either extremity. In addition to spiral movements, the whole body of the spirochæte often shows undulations, which are particularly evident in some of the free-living water forms. In the sub-genus *Leptospira* there is such a close spiral that a peculiar type of movement results, and the ends of the organism are frequently bent in the form of a crook, which may produce the optical illusion of a terminal swelling as a result of the very rapid rotations.

The manner in which movement is produced is not very evident, but according to Noguchi (1928) is dependent on the axial filament. This is supposed to be a spring-like structure, analogous to a bacterial flagellum, which constitutes the framework of the spirochæte and is surrounded by a layer of contractile protoplasm enclosed in a delicate periplast. When suspended in an isotonic medium the pressure of the contractile protoplasm stretches the axial filament and thus produces a rotary movement. Alternate contractions and relaxations produce movement in one direction or the other. By means of this firm axial filament spirochætes are able to bore through solid or semi-solid media.

The flexibility of the body of spirochætes is a very constant feature and serves to distinguish them from the *Spirilla*, with which they have often been confused.

Size.

The dimensions of spirochætes vary from giant forms like *S. plicatilis*, with a length of 500μ and a diameter of 0.75μ , down to *S. minima*, 2μ in length and 0.2μ in diameter, whilst some of the *Leptospira* are even finer, and can only be detected with the aid of dark-ground illumination. It should be emphasized that under certain conditions very long forms are produced, either by the suppression of transverse division, or as a result of a number of individuals becoming attached together by their extremities. It will be realized, therefore, that the length of any particular species is subject to considerable variation and it is necessary to measure a considerable number of individuals (100 or more) in order to arrive at any reasonable estimate of the mean length. The number of turns of the spiral is also of significance, some species having a much closer spiral than others.

Certain authors have devised methods of measuring the curvature of the spirals, and obtaining figures indicating the relation between the depth and length of each individual turn of the spiral. de Mello and de Andrade (1922) divide the sum of the lengths of 100 organisms by the total number of turns they possess and thus obtain a figure indicating the average length of each spiral. Achitouv (1924) has elaborated a mathematical formula for the measurement of the length of each curve of the spiral, and Delamare and Achitouv (1925) claim that this index of curvature gives an indication of the morphological type of spirochæte under observation. In the opinion of most authors, however, it is not possible to determine the species by these methods alone.

Nomenclature and Classification.

Apart from the type of movement, the mean length, the index of curvature, and the diameter, most pathogenic spirochætes have no other distinguishing morphological characters, and it is hardly necessary to state that, as in the case of most bacteria, morphological characters alone are quite inadequate to distinguish the majority of forms, and it is necessary to employ the so-called biological methods, which are really physiological.

The author is well aware that the Linnæan system of classification is based entirely on morphological distinctions, and that there is some objection to using the same method of nomenclature for organisms distinguished mainly by physiological differences. This method is generally used in bacteriology, however, and as long as it is understood that these so-called species do not correspond with species in the Linnæan sense, it seems more convenient to retain a method which has been generally adopted in the past. Otherwise, all the pathogenic spirochætes and many

free-living forms would be reduced to three or four species, which, however commendable from a strictly taxonomic point of view, would certainly not appeal to the pathologist, whose main interest is in the biological properties of organisms.

Classification here Adopted.

The general method of classification adopted in the following pages is essentially that proposed by Dobell (1912¹), with the exception that the author agrees with Mesnil (1921), Zuelzer (1925) and the majority of continental authorities, including all the contributors to the *Handbuch der pathogenen Mikroorganismen* (Kolle, Kraus and Uhlenhuth, 1930), that in the present state of our knowledge it is impossible to draw a clear line of distinction between the free-living water spirochætes and the pathogenic forms. Consequently all known pathogenic spirochætes, without exception, are referred to one genus, *Spirochæta*, including *Treponema* and *Leptospira*.

Family Spirochætaceæ.

Spiral, filamentous and highly flexible organisms. Multiplication by transverse division usually into two daughter-individuals, but sometimes into three or more. The apparatus of locomotion consists of an elastic axial filament or a modified membrane along the side of the body, and alternate contractions and relaxations of the contractile substance surrounding the axial filament serve to propel the organism in the direction of either extremity.

Genus 1. Spirochæta. Ehrenberg, 1834. (Synonyms—*Spiroschaudinna*, *Borrelia*, *Spironema*, *Microspironema*, *Treponema*, *Leptospira*.) Free-living or parasitic Spirochætaceæ with the general characters of the family, but distinguished from the other two genera by the absence of a chambered structure. The larger forms show the presence of an axial filament. The type species is *Spirochæta plicatilis* (Ehrenberg, 1834) (Figs. 1, 2, 7-14, & 18-25).

Genus 2. Cristispira. Gross, 1910. Spirochætaceæ living in the crystalline styles or digestive tract of lamellibranchs. They are characterized by the possession of a crista, a fine membrane about the width of the body, which seems to be an extension of the periplast in the form of a crest or ridge running spirally along the length of the body. In addition the body is divided into chambers by means of transverse septa. Division is transverse and sporulation has also been described. The extremities are blunt. The dimensions vary from 45 to 90 μ in length by 1 to 1.5 μ in diameter. There are 2 to 5 very open spirals or undulations. The crista quickly disappears when exposed to the action of tryptic digestion or 10 per cent. bile-salts, and becomes indistinct in 10 per cent. saponin. Distilled water produces general plasmolysis and disintegration. The type species is *Cristispira balbianii* Certes, 1882 (Figs. 3-6).

Genus 3. Saprospira. Gross, 1911. Free-living saprophytic forms occurring in foraminiferous sand. They resemble *Cristispira* in having

the body divided into chambers by transverse septa, but differ in the absence of a crista. Multiplication is by transverse division and sporulation has been recorded. The extremities are blunt. The length varies from 100 to 120 μ and there are no definite spirals, but only two or three inconstant curves. The organism moves by undulations of the body. The type species is *Saprospira grandis* Gross, 1911.

Noguchi's Classification.

Opinions differ, however, as to the desirability of including the large free-living spirochætes in the same genus as the various pathogenic forms, and Noguchi (1928) has proposed to subdivide the genus *Spirochaeta* as constituted above, into three distinct genera: *Spirochaeta* (*sensu stricto*), *Treponema* and *Leptospira*, whilst retaining *Saprospira* and *Cristispira*. According to his classification the characters of the genera are as follows:

Genus Spirochaeta. Ehrenberg, 1834. Flexible organisms with an indistinct membrane, consisting of a straight axial filament surrounded by a layer of alveolar protoplasm coiled spirally around the axial filament. Volutin granules arranged in pairs at each turn of the spirals. Multiplication by transverse and multiple division. All known forms are free-living in fresh or salt water. The extremities are often rounded. The length varies from 100 to 500 μ and the diameter from 0.5 to 0.75 μ . The spiral amplitude is 2 μ and spiral depth 1.5 μ . The number of spiral turns varies from 50 to 250. The axial filament is distinctly seen in stained specimens. Under the influence of tryptic digestion all parts are dissolved except the axial filament; 10 per cent. bile-salts render the body pale and shadowy, but it does not dissolve; in 10 per cent. saponin *S. plicatilis* lives 30 minutes, then becomes shadowy but does not dissolve. Distilled water produces plasmolysis. The movements are slow and creeping and the spirals remain unchanged.

Type species: *S. plicatilis* Ehrenberg, 1834, a large free-living saprophytic spirochæte found in stagnant water, especially water containing sulphuretted hydrogen.

Genus Treponema. Schaudinn, 1905. These organisms are relatively long, cylindrical and highly flexible, with sharply pointed extremities. The movements may be serpentine, or corkscrew-like, sometimes with lashing of the extremities. The spirals are alternately stretched and relaxed especially in very active specimens, but in less active ones the spirals are more rigid, and the organism may rotate along its axis first in one direction and then in the other. The average dimensions range from 8 to 14 μ in length by 0.25 to 0.3 μ in diameter; the spiral amplitude is 1 μ and spiral depth 0.8 to 1 μ and very constant. The number of spiral turns, therefore, ranges from 8 to 14. The axial filament can be seen in degenerating examples of some species, and according to some views the whole body consists of an axial filament with a thin covering of protoplasm. No differentiation of structure can be detected in ordinary Giemsa-stained specimens, the whole body staining either red or reddish

violet according to the thickness of the protoplasm surrounding the (hypothetical) axial filament. The more protoplasm the greater the tendency for the organism to take up the violet tinge. Multiplication is by transverse division, and in addition a granular phase has been observed in a number of forms. The organisms withstand tryptic digestion for many days ; in 10 per cent. bile-salts the axial filament of the coarser forms is exposed and finally the whole body is dissolved. Ten per cent. saponin and also distilled water immobilizes them in 30 minutes, and after a few hours they are broken up ; this constitutes one of the most striking differences between *Treponema* and *Leptospira*, as the latter are completely resistant.

The genus *Treponema* as defined by Noguchi, thus includes both the tissue-inhabiting forms such as *S. pallida*, requiring anaerobic conditions for growth, and the blood spirochætes such as *S. recurrentis*, which are aerobic. The blood spirochætes are often distinguished generically from the tissue spirochætes (*Treponema sensu stricto*) and if that method is adopted, the generic name *Borrelia*, suggested by Swellengrebel (1907), would be their correct designation.

This genus includes the great majority of the pathogenic spirochætes. The type species is *Treponema pallidum* Schaudinn, 1905, the causative organism of syphilis.

Genus Leptospira. Noguchi, 1917. The body is long, slender and cylindrical, with sharply pointed extremities, but the spirals are very tightly set, regular and more shallow than in the genus *Treponema*. The average length is about 7 to 14 μ and diameter 0.25 to 0.3 μ . The spiral amplitude is 0.45 to 0.5 μ and depth 0.3 μ , and the number of spiral turns varies from 14 to 24 ; these do not change their curves in the active state, but may become unwound when the organism is dying. A smooth axial filament with hooked ends may be present according to Noguchi, but cannot be distinguished by staining. The terminal portions are much more active than the middle part of the body, often lashing about and rotating so rapidly as to produce the optical illusion of a terminal loop (Fig. 19). They are easily dissolved by tryptic digestion and 10 per cent. bile-salts, but withstand the action of 10 per cent. saponin and distilled water. They are all obligatory aerobes.

This genus includes a number of saprophytic and pathogenic forms which closely resemble each other. The type species is *Leptospira icterohæmorrhagiæ* Inada and Ido, 1915.

Reproduction.

It is now generally agreed that the only ordinary method of multiplication is by transverse division, the details of which vary slightly in different forms ; some multiplying by simple division into two, and others by multiple division into three or more daughter-forms. In the very great majority of pathogenic forms simple division into two seems to be the

only method. When a spirochæte is about to divide, the only change observed is a slight constriction about the middle of the body ; the two halves gradually become drawn apart, but often for some little time a very fine connection prevents the final separation of the daughter-individuals (Fig. 14). In the last stage of division the two halves may get twisted together and the subsequent unrolling and final separation is difficult to distinguish from longitudinal division, not only in the living state, but also in stained preparations. The existence of such forms, combined with the influence of Schaudinn's original description, is doubtless responsible for the many accounts of longitudinal division in earlier publications, but with more careful observations the view that this method of reproduction takes place has now been generally abandoned. In some species, such as *S. eurygyrata*, division may be unequal, resulting in the production of short and long forms, and in cultures of these organisms it is common to find all stages between short forms, resembling *Vibrios* and typical spirochætes (Hogue, 1922).

The life-history of spirochætes is by no means decided and a variety of explanations, descriptions and hypotheses have been put forward to account for the real or, according to some authors, only apparent disappearance of spiral forms at certain stages in the development of these organisms. The various theories will be briefly considered, but at present the problem must still be regarded as *sub judice*.

1. *Disappearance of spirochætes only apparent, not real.*

This is a somewhat difficult view to attack, for these organisms, especially if present in small numbers, are very easily overlooked. Thus the earlier view that in animals infected with relapsing fever, the spirochætes as such disappeared from the blood in the intervals between successive attacks, is now questionable, as further observations have shown that the organisms may persist in very small numbers, so few as to render the chances of finding them in ordinary smears very remote. By the use of thick films, however, it has been possible to demonstrate the presence of spirochætes in the intervals between successive attacks.

When the spirochæte passes through an intermediate host, for instance *Spirochaeta recurrentis* in the body louse, *S. anserina* in *Argas persicus*, &c., the disappearance of the organisms may again be only apparent, for if two or three spirochætes alone persisted in the body of a tick, they might be sufficient to carry on the infection, though the chances of their detection by ordinary microscopical methods would be very small. Consequently, although there is a fairly general agreement on the part of most investigators of the problem, that after being ingested by the transmitting louse or tick, spirochætes, as such, disappear at any rate for a time, the possibility of odd ones persisting cannot be definitely excluded.

It is possible, however, to exclude the persistence of spiral forms in the eggs laid by *Argas* infected with *S. anserina*. In the case of this disease, the infection is transmitted hereditarily to the offspring of an

infected tick (Hindle, 1912¹) and the eggs can be proved to contain the infection by inoculation into susceptible birds. It is not very difficult to examine the contents of a tick's egg, and definitely exclude the presence of spirochætes, yet the author has infected birds with spirochætosis by the inoculation of the contents of eggs that were negative to microscopic examination (Hindle, 1912²).

Unfortunately, the issue is again complicated by the undoubted fact that at certain temperatures spirochætes, as such, may be found in the eggs laid by infected ticks. This question will be referred to later (*vide infra*, p. 178).

2. *Disappearance of spirochætes caused by their attenuation.*

This view was originally advanced by Prowazek, who noted the disappearance of spirochætes from the gut of *Argas* that had been fed on fowls infected with *S. anserina*. Prowazek (1909) explained the disappearance as the result of repeated longitudinal division, whereby the spirochætes became finer and finer until they were no longer visible. It is doubtful whether this explanation would find any supporters at the present time, for the belief in the occurrence of longitudinal division has been generally abandoned.

Essentially a modification of the same view has been advanced by Marchoux and Couvy (1913), who consider that spirochætes are continually present in the tissues of infected *Argas*, but in starving ticks become so fine that they cannot be detected by ordinary staining methods. According to these authors the thickness of the spirochætes diminishes when the ticks are starved, but the normal dimensions are recovered after the ticks have ingested blood.

It is possible that the staining properties of the spirochætes may have been affected by variations in the media in which they were living, for it is difficult to believe that these organisms vary in diameter in this manner.

3. *Disappearance of spirochætes caused by change in form.*

Numerous authors from Dutton and Todd (1907) onwards have observed that under certain conditions spirochætes divide up into granules, or coccoid forms, and it is a very difficult question to decide whether these granules represent a method of reproduction, or are merely the result of degeneration. In the case of *S. duttoni* Leishman (1920) has described stages in the development of the granules into spirochætes, and similar observations have been recorded by Balfour (1911) (Figs. 20–22), Fantham (1911) and Hindle (1912³) in the case of *S. anserina* the fowl spirochæte. Sergeant and Foley (1914), Nicolle, Blaizot and Conseil (1913) and Nicolle and Lebailly (1920) also note the complete disappearance of spirochætes in lice for varying intervals after being fed on blood containing *S. recurrentis*. Nicolle (1927), as a result of observations on various relapsing-fever spirochætes, states that there are three stages, indifferent granules, previsible and infective forms, and the adult spirochæte, which is the ancestral saprophytic type.

There is a considerable body of evidence, therefore, to show that spirochætes must have a phase in which they are not recognizable as such, and at present the most reasonable hypothesis is the one first developed by Leishman, that the granules or coccoid bodies represent a stage in the development of these organisms.

It is universally admitted that when ingested by their invertebrate host, spirochætes do break up into these granules and the absence of spirochætes, as such, from infected tissues (e.g. the eggs laid by infected ticks, the contents of infected lice and ticks), all support the view that some other phase exists. The widespread occurrence of granules, some of which are organisms of the *Rickettsia* type, in the cells of Arthropods, renders it difficult to distinguish any particular type of granule, and if the spirochætes had assumed this phase the uncertain findings in infected animals would be explained.

The allied genera *Saprospira* and *Cristispira*, which, being considerably larger are more easily studied, show, according to Gross (1912), the formation of spores (Figs. 3 & 4), therefore it is inherently probable that members of the genus *Spirochaeta* also have a similar stage. Confirmatory evidence in support of this view is also afforded by the examination of cultures of spirochætes. Martin and Pettit (1919) observed granular forms in cultures of *S. icterohæmorrhagiæ*, and Noguchi (1919) infected guinea-pigs with cultures containing only granular forms of this organism, the spirochætal stage appearing in the blood and organs of the animals. Similar results were also obtained by Noguchi (1913) in the case of *S. pallida*. Moroder (1929), working with four strains of relapsing fever, found that cultures which were completely negative to microscopic examination for spirochætes produced infection when inoculated into mice, or showed spirochætes in subcultures. Often these seemingly negative cultures showed small granules, resembling micrococci, but cultures on agar slopes showed that they were not contaminating organisms.

Recently, Seguin (1930) has described the breaking up of *Spirochaeta calligyra* into minute granules each with a spiral filament by means of which they are able to move about. This filament can only be seen after special staining methods and is not visible under dark-ground illumination. All stages in the development of these forms into ordinary spirochætes have been observed. Manouélian (1930) has also observed similar stages in tertiary lesions of syphilis, and thus the view first put forward by Balfour (1911) is confirmed. It is of interest that Knowles, Gupta and Basu (1929) (see below, p. 179), also found short spirochætes with a terminal flagellum in *Argas* infected with *S. anserina*, therefore it is possible that the developmental stages of other spirochætes may show a flagellum.

General Conditions of Life.

The free-living spirochætes can obviously withstand considerable variations in temperature as they occur in waters that are frozen in winter and warmed in summer, but cultures of ordinary water leptospira

grow best at temperatures between 20 and 30° C. and above 40° C. are soon killed. Although all known pathogenic spirochætes are killed when the temperature rises above 50° C., a saprophytic form, *Spirochaeta daxensis*, lives in the hot springs of Dax at a temperature of 56° C. (Cantacuzène, 1910). Most spirochætes seem to resist long exposure to cold, for relapsing-fever spirochætes in citrated blood kept at 0 to 2° C. remained alive up to 105 days (Nagão, 1923), and cultures of *S. pallida* can withstand long exposure to temperatures of -16 to -20° C. (Kissmeyer, 1923; Krantz, 1923).

Moisture is essential to the life of all known spirochætes, none of which are able to withstand desiccation at any stage in their development. Consequently the pathogenic forms cannot be air-borne and infection must depend either on direct contact, or water, or the agency of transmitting intermediate hosts.

Some of the saprophytic forms can withstand considerable variations in osmotic pressure, for Uhlenhuth and Zuelzer (1922) found spirochætes living in water containing 4.5 per cent. sodium chloride.

The hydrogen-ion concentration has a marked effect on the growth of spirochætes, most of them requiring a slightly alkaline medium. As a result of this susceptibility, acid waters are generally free from these organisms.

When grown in cultures some spirochætes require oxygen, whilst others are anaerobic (see p. 112). Although Noguchi's views have been adopted on this question, it should be noted that the oxygen requirements of the so-called aerobic group are extremely small and it is probable that most of them can persist under anaerobic conditions.

The influence of carbohydrates on the growth of these organisms is another debatable question, for although Akatsu (1917) found that they had no effect on the growth of *S. pallida*, *S. calligyra*, &c., and even an adverse effect on the growth of aerobic forms, other observers have obtained different results. Ponselle (1910) found that when the blood of a fowl containing *S. anserina* was preserved *in vitro*, the spirochætes soon agglutinated and became immobile, this corresponding with the disappearance of the blood-sugars. If glucose was then added to the blood the spirochætes recovered their motility. The spirochætes are unable to assimilate glucose directly, but require a catalytic agent which seems to be present in the blood. Bruynoghe and Dubois (1927) have also found that sugars are essential to the metabolism of blood spirochætes, especially for movement. The addition of glucose, lævulose, maltose, galactose and dulcitol has a very favourable action in restoring the motility of spirochætes in blood deficient in sugar; lactose, saccharose and glycogen have a moderate effect; mannitol a slight effect; and dihydroxyacetone and glycerol are inactive.

✓ The influence of ordinary light on spirochætes seems to be negligible. On the other hand, blood spirochætes exposed to the action of β and γ rays for periods up to 47 hours were still infective, but exposure to the

emanations for 5 hours destroyed their infective powers, although their motility remained unaffected (Bruynoghe and Dubois, 1925).

Similar results were also obtained by Zuelzer and Philipp (1926) working with strains of *S. icterohæmorrhagiæ* and *S. recurrentis*.

Cultivation.

The future study of spirochætes, more especially of the pathogenic forms, must be largely dependent on their successful cultivation, as in many cases this affords the only practicable method of separating the various species living together with ordinary bacteria in the same parts of the body.

According to their cultural requirements, Noguchi (1928) divided spirochætes into two groups, one aerobic and the other anaerobic. The aerobic group includes the blood spirochætes and leptospira, and the anaerobic group, the tissue spirochætes and various saprophytic species. As a general rule the former are more easily cultivated, especially members of the leptospira group. *S. icterohæmorrhagiæ*, the first of these to be cultured, was grown by Inada and his fellow workers in 1916. They used ascitic fluid containing fresh tissues and found that the optimum temperature was 22 to 25° C.; under these conditions cultures persisted much longer than others grown at 37° C. Reiter and Ramme (Reiter, 1916) found that the same organism would grow well in a mixture of one part rabbit-serum and five parts of 0.85 per cent. saline solution, and subsequently various modifications of dilute serum have been generally used for the cultivation of leptospira. According to Noguchi (1928), rabbit-serum is preferable to all other animal sera, with the possible exception of sheep, as it gives a far richer growth of organisms. The presence of semi-solid particles, such as pieces of agar, also favours growth, and therefore the best results are obtained in rabbit-serum media containing sufficient nutrient agar to render them semi-solid. Since the organisms are obligatory aerobes the agar must not be too solid, otherwise oxygen cannot easily penetrate to any depth and the growth of the cultures is inhibited. A concentration of 0.1 to 0.3 per cent. agar furnishes the optimum conditions and allows the penetration of oxygen to a depth of 2 to 3 cm.

The medium recommended by Noguchi for the routine cultivation of all leptospira is as follows :

	<i>Parts.</i>			
0.9 per cent. sodium chloride	800			
Rabbit-serum	100			
Hæmoglobin solution	0.1 to 0.2			
2 per cent. nutrient agar	100			

The agar is melted, cooled to about 45° C. and added quickly to the saline, serum and hæmoglobin mixture, which has been warmed to the same temperature. Thorough mixing is important, as a uniformly gelatinous medium gives better results than one containing flocculent particles. The hæmoglobin is prepared by mixing one part of defibrinated

rabbit's blood with three parts of distilled water. It is not essential to the growth of leptospira, but if the medium is used for other organisms, such as *Leishmania* or *Bartonella*, its presence is desirable. A layer of paraffin oil may be added to the culture tubes to prevent the evaporation of the medium, but if transfers are made every two months this is unnecessary.

In addition to the above, other methods of cultivating these organisms are referred to in Chapter IX.

The anaerobic group includes the first spirochætes to be cultivated, namely *Spirochæta vincenti*, grown by Tunncliffe (1906) on a glucose-agar medium with or without the addition of serum. *S. dentium* from the human mouth was also cultivated by Mühlens and Hartmann (1906) on a similar medium, and other mouth spirochætes grown at 37° C. by Repaci (1911) in Veillon's medium.

The most important advance in our knowledge of this subject was afforded by Noguchi's cultivation of *Spirochæta pallida* in 1911. This method consists of incubating material containing the organisms in the presence of fresh tissue under a tall column of ascitic fluid or sheep-serum water. The cultures are grown at 37° C. under anaerobic conditions. Various modifications of this method have been used with success for the isolation and growth of some of the tissue spirochætes and saprophytic forms, of which Noguchi (1928) has given a useful summary.

The direct isolation of the parasite from contaminated material is effected by the use of a solid medium prepared as follows: A rabbit kidney is removed with sterile precautions and cut into about 16 pieces. A piece of this tissue is placed into each of a number of tall narrow test-tubes (14 to 20 × 200 mm.). Two parts of nutrient agar are then mixed with one part of ascitic fluid, the mixture being kept at about 50° C., and 15 c.cm. is poured into each of the tubes containing the sterile tissue. The medium is allowed to cool and after solidification the material containing the spirochætes is inoculated at the bottom of the tube either by means of a platinum loop or capillary tube. A layer of liquid paraffin is added to prevent evaporation and the tubes incubated at 37° C. for 2 to 3 weeks in the case of *S. pallida*, or about one week if saprophytic forms are being grown.

The ordinary bacteria grow up along the stab canal and on the surface of the medium, but the spirochætes penetrate into the agar, and a slight haze, caused by their presence, can sometimes be seen radiating from the stab canal. When growth has taken place the surface of the medium is sterilized by pouring on sublimate alcohol, and the culture-tube is broken about the middle, by making a scratch with a diamond and applying a red-hot glass rod. The upper half of the broken tube is then drawn off, exposing the column of agar, which is again sterilized by washing with sublimate alcohol, care being taken to dry off the excess moisture by means of sterile gauze. The agar column is then bent until it breaks across and exposes a surface on which the colonies of spirochætes, if present, can be seen as hazy regions. By means of a sterile capillary

pipette material from these areas can be removed and if the presence of spirochætes is confirmed by dark-ground examination, it can be used for subinoculation into similar culture-tubes. After a varying number of subcultures the spirochætes may finally be obtained free from contaminating bacteria.

Employing this method various spirochætes have been isolated, including *S. pallida*, *S. pertenuis*, and various saprophytic forms, especially from the mouth and genitalia, including *S. macrodentium*, *S. refringens*, *S. mucosa*, *S. calligra*, *S. genitalis*; also *S. phagedenis* from phagedenic ulcers.

Once the spirochætes have been obtained in pure culture they can then be grown in a fluid medium prepared in the same way as the solid one, except that ascitic fluid or serum water (one part rabbit-, sheep- or horse-serum and three parts distilled water) is added to the tubes containing sterile kidney, instead of the agar mixture. It is advisable to introduce the material containing spirochætes together with the kidney, before the addition of the fluid. Finally a layer of liquid paraffin is added and the tubes placed in an anaerobic jar. They are incubated at a temperature of 37° C. and left undisturbed for 2 to 3 weeks. If the growth is impure it is sometimes possible to purify the cultures by the use of a filter, as the spirochætes will grow through a filter more quickly than ordinary bacteria. As a general rule, however, the use of this fluid medium is only recommended for the growth of pure cultures, the solid medium being more suitable for impure material. Moreover, the solid medium is more satisfactory for the maintenance of cultures in the laboratory as with it the use of anaerobic jars is unnecessary. The requisite anaerobic conditions are produced by the tissue and the entrance of oxygen is prevented by the column of solid agar.

It should be emphasized that only a comparatively small proportion of original cultures are successful, but once obtained the cultures can be subcultured without much difficulty and the cultural requirements of the organisms seem to become less exacting so that after several passages they may be grown in other media. The virulence of *S. pallida* is very soon lost in cultures, and it is evident that neither of the media referred to furnishes ideal conditions for the growth of tissue spirochætes.

Blood spirochætes were first cultivated by Noguchi (1912) in ascitic fluid containing fresh tissue incubated at 37° C. under aerobic conditions. The conditions for their growth in general resemble those outlined above for leptospira, but if a semi-solid serum-agar medium is used, the hydrogen-ion concentration must be carefully adjusted to a pH of 7.4 to 7.8 (Kligler and Robertson, 1922). According to some authors, e.g. Lapidari and Sparrow (1928), the growth is favoured by anaerobic conditions, but the presence of a covering layer of paraffin oil does not prevent the entry of oxygen into the surface layers of the medium as originally suggested by Noguchi. The general conditions for the growth of this group of spirochætes are discussed in Chapter VI.

Filterability.

In this property spirochætes range from easily filterable forms such as the members of the leptospira group, through others more difficult to filter, such as the blood spirochætes, to the tissue spirochætes which are not directly filterable.

The blood spirochætes were the first members of the group shown to be filterable, Novy and Knapp (1906²) obtaining infection by the inoculation of the filtrates of blood from animals infected with the American strain of relapsing fever, and Breinl and Kinghorn (1906) obtaining similar results with an African strain, *S. duttoni*. In both series of experiments Berkefeld filters were used and the authors' descriptions suggest that the filtrates were completely negative to microscopical examination. Todd and Wolbach (1914), working with four strains of African relapsing fever, obtained filtration through No. 9 Berkefeld filters, either N, W or V, but only when the pressure was increased to 50 pounds per square inch. These authors were of the opinion that actual spirochætes passed through the filter and were responsible for the infectivity of the filtrate.

The members of the leptospira group will readily pass through Berkefeld filters, as first shown by Wolbach and Binger (1914) in the case of the common water leptospira, *S. biflexa*. These results have been repeatedly confirmed, and both the pathogenic and free-living forms will pass through Berkefeld filters or Chamberland filters as fine as grade L3 (Hindle, 1925). The use of filters, usually Chamberland L3 or a similar grade, thus affords a ready means of obtaining these organisms in pure culture. Here again there is some doubt as to the form which is in the filtrate, some authors being of the opinion that actual spirochætes pass through the filter, whilst others, having obtained uniformly negative results by the microscopical examination of the filtrate, consider that the spirochæte is present in some other phase.

The tissue spirochætes *S. pallida* and *S. pertenuis*, according to Noguchi (1928), are unable to pass directly through ordinary filters, but can grow through the pores of a Berkefeld filter (V or N) in about five days, if provided with suitable cultural conditions.

Pathogenic Action.

Spirochætes present an interesting series of stages in the development of a parasitic mode of life from organisms that normally are free-living, but under certain conditions may become parasitic, up to exclusively parasitic forms such as *Spirochæta pallida*. Most fresh and salt waters contain saprophytic spirochætes closely resembling the pathogenic forms in their morphology, and in some cases these have been shown to be capable of becoming pathogenic. The best-known example is the ordinary water leptospira, which may be cultured from practically any river or tap-water. Repeated experiments have shown that this organism, under certain ill-defined conditions, may live in the body of man or experimental

animals, and produce varying harmful effects ranging from mild fever up to fatal hæmorrhagic jaundice.

Uhlenhuth and Zuelzer (1922) found that a water strain of *Spirochæta* (*Leptospira*) *icterohæmorrhagiæ*, after being cultivated in serum media for 15 months, became pathogenic to guinea-pigs. Later, Baermann and Zuelzer (1928) gave particulars of nine strains of free-living leptospira, all of which had been made pathogenic in various ways. For example, 2 c.cm. of a fresh culture of one of these strains were inoculated (subcutaneously, intracutaneously and intravenously) into a woman, who after 4 days showed a slight rise in temperature, which persisted for 6 days without any other clinical signs. Blood taken from the patient on the sixth, seventh and eighth days and inoculated into a guinea-pig was followed by the development of typical jaundice in this animal after an incubation period of 26 days. This animal was killed and examined for leptospira, but none found; its organs, however, showed typical lesions of Weil's disease. The blood and organs were inoculated into three other guinea-pigs and cultures made from the heart-blood. All three animals showed leptospira on the third day and died on the sixth day with typical symptoms of Weil's disease. The patient's serum agglutinated and dissolved the original water strain but had no effect on the strain after passage through guinea-pigs or on other strains of leptospira. Therefore, passage through two animals had completely changed the nature of this spirochæte, and the change persisted.

It should be noted that these results are not generally accepted, many authorities attributing these infections either to the existence of unrecognized pathogenic strains mixed with the original water strains, or to possible contamination of the guinea-pigs by the agency of rats, which are well known to pass out virulent leptospira in their urine for many months after having been infected. Rats could certainly contaminate food and water, but it is difficult on this hypothesis to explain the results of the above-mentioned experiments.

In addition to the free-living water spirochætes there are a very large number of normally saprophytic species, inhabiting the bodies of man and animals, which may also acquire pathogenic properties. The mouth, alimentary canal, and also the healthy genitalia of man, often contain spirochætes closely resembling pathogenic forms, and it is probable that by mutation and adaptation they may, like the water forms, be able to become transformed from harmless saprophytes into dangerous parasites.

It is possible that the numerous cases of bronchial spirochætosis recorded from various parts of the world constitute an example of normally saprophytic mouth spirochætes becoming pathogenic; and also various ulcerous conditions of the alimentary canal often contain large numbers of these organisms. In most of these cases, however, there is no clear evidence indicating that the spirochætes are directly responsible for the pathological conditions, for hitherto animal experiments have given rather inconclusive results.

The intestinal spirochætes seem able to invade the blood under certain pathological conditions, but this property is shared by other intestinal organisms, and usually only occurs towards the death of the host.

Sanarelli (1927), who was the first to record this phenomenon, has made a careful study of these cæcal parasites, which were twice obtained in pure culture, on one occasion from the heart-blood, and on the other from the spleen of guinea-pigs kept at a temperature of 37° C. According to this author, spirochætes in general have little direct pathogenic action, but their presence predisposes the development of secondary infections, as a result of the direct or indirect action of their proteins. For example, if these cæcal spirochætes are inoculated into the anterior chamber of the eye of a rabbit, an intense iridocyclitis is produced, but the aqueous humour is unaffected and after 5 or 6 days the eye becomes normal. If at the same time a culture of *Staphylococcus aureus* is injected into the other eye, within 3 days this organ develops an intense panophthalmia which continues until it is destroyed. After 12 to 18 days the eye which received the injection of spirochætes suddenly develops a violent iritis, followed by the development of panophthalmia and destruction of the organ. The contents of this eye are found to contain no organisms except *Staphylococcus*, all trace of spirochætes having disappeared. It would seem, therefore, that the original presence of these spirochætes, or their proteins, favoured the subsequent development of a suppuration process.

The manner in which spirochætes may favour the development of other infections was also demonstrated by the subcutaneous inoculation into a rabbit of a mixture of these organisms with a culture of *Staphylococcus*. Under these conditions the rabbit often died in 24 hours, whilst the inoculation of staphylococci alone produced merely a localized suppuration which healed within 20 days, and of the spirochætes alone, produced no obvious effect.

Sanarelli has extended these observations from the supposedly saprophytic spirochætes of the alimentary canal to the relapsing fever organisms and leptospiræ. According to his view most spirochætes are as a rule only harmless parasites, or commensals, as, for example, the spirochætes found in the alimentary canal. However, in contact with altered tissues, with a lowered resistance, or when associated with certain secondary organisms, especially proteolytic ones, these commensals may become pathogenic, or at least favour the development of other infections.

Vincent's angina, bronchial spirochætosis, and phagedenic ulcers, are possibly the result of such joint action on the part of spirochætes and other secondary organisms.

The relapsing fever spirochætes are exclusively parasitic and are found in the blood of vertebrates. They are probably all transmitted by the agency of some intermediate host, in which the spirochæte can persist indefinitely. Although parasitic, some of these spirochætes have very

little pathogenic action and the various strains of human relapsing fever, which are indistinguishable except by physiological tests, show great differences in their pathogenicity.

Spirochæta pallida is a good example of a spirochæte which has become adapted to an exclusively parasitic mode of life, and also is highly pathogenic. The group to which it belongs seems to inhabit the tissues of mammals, and infection is transmitted from one host to another by contact. These organisms also show great differences in their pathogenic action, without any corresponding morphological distinction, and it is impossible to distinguish *S. pallida*, *S. pertenuis* and *S. cuniculi*, although they produce three distinct diseases, namely human syphilis, yaws, and rabbit syphilis respectively.

The pathogenic spirochætes, for convenience, may be divided into four groups, according to the type of infection produced by them.

1. *Commensal spirochætes, including the muco-cutaneous group.*

Spirochætes found in association with other bacteria, inhabiting various superficial ulcerative conditions. The pathogenicity of these forms is somewhat doubtful, for they seem to be rather in the nature of saprophytes living in tissues which have already become diseased, but, as indicated above, they may favour the development of secondary infections. Their presence is very constant in such affections as Vincent's angina, bronchial spirochætosis, and in certain chronic ulcers.

2. *Blood spirochætes.*

Spirochætes normally inhabiting the blood of man and animals, in which they generally produce a relapsing type of fever. These infections are transmitted from one host to another by the agency of some blood-sucking arthropod, such as ticks or lice. European relapsing fever, tick fever and fowl spirochætosis, are well known examples of this group.

3. *Tissue spirochætes.*

Spirochætes which invade the tissues and generally produce a very chronic type of infection. Transmission from one host to another, as a rule, seems to be the result of contact with an infected surface, such as a mucous membrane or ulcer. The spirochætes of this group used to be commonly known as *Treponema* (*sensu* Schaudinn) and the best-known diseases caused by them are syphilis, yaws, and rabbit syphilis.

4. *Leptospira.*

Spirochætes which generally invade the kidneys of man and animals, although other organs, such as the liver, are frequently involved. The organisms pass out of the body in the urine, and transmission seems to be usually effected by contamination of water. Some form of jaundice is a feature in the group of diseases caused by these organisms, of which spirochætal jaundice, or Weil's disease, is the best-known example.

Immunity.

Natural Immunity.

Many races of spirochætes are restricted to a few species of hosts, and all other animals are resistant.

S. pallida, for example, produces typical infections only in primates, although certain monkeys and rabbits can also be infected experimentally. The relapsing-fever spirochætes show considerable individual differences in their infectivity towards hosts other than their natural one, some races being capable of living in a great variety of hosts, whilst others are very specific. As a general rule, however, the human relapsing-fever spirochætes will only develop readily in monkeys, and rats, mice, or other small rodents. Most other animals are resistant to the infection. Many species of birds have been shown to be susceptible to infection with the fowl spirochæte, *S. anserina*, but other vertebrates are naturally resistant to infection. In the case of *Spirochæta* (*Leptospira*) *icterohæmorrhagiæ*, the guinea-pig is peculiarly susceptible to infection, whilst many of the ordinary laboratory animals are more or less resistant. These animals are not resistant because of immune-bodies present in the serum, for the spirochætes will live for hours in the serum of monkeys, rabbits, mice, &c., but when the organisms are mixed with blood intense phagocytosis takes place and results in their destruction. On the other hand, it is possible to infect rats and mice by subcutaneous inoculation, for the spirochætes find their way to the kidney and in some manner evade being destroyed for long periods. Such animals are a source of danger as animal reservoirs, for the spirochætes inhabiting the kidney are passed out with the urine, in a form which is fully virulent to susceptible hosts, such as guinea-pigs, and presumably, man.

The age of the animal seems to be a factor in natural immunity to certain spirochætal infections, for old fowls are much more resistant to infection with *S. anserina* than young chicks. On the other hand, Marchoux found that after several passages through young chicks the spirochæte lost its virulence for fowls. Anderson (1928), however, has shown that the virulence of a strain may be restored by inoculation into eggs that have been incubated for 15 days, or into two-days' old chicks.

As would be expected, dosage is an important factor and also the site of inoculation; in the case of *S. anserina*, Marchoux and Couvy (1913) found that whereas intravenous inoculation of only 70 spirochætes into a Padda bird immunized it against a subsequent dose, an intramuscular inoculation required about 2,000 and a subcutaneous, 1,700 to 7,353 organisms to produce the same result. When inoculated subcutaneously, 700 spirochætes had no effect, 1,700 vaccinated the bird against a subsequent dose, and 20,000 were required to produce a recognizable infection. The influence of dosage has also been shown in the case of mice inoculated with *S. recurrentis*.

Acquired Immunity.

Recovery from spirochætal infections is generally followed by the development of two kinds of immunity, one depending on the persistence of a latent infection, and the other on the development of immune-bodies against the organism.

Immunity and the 'Carrier' Condition.

This is a type of immunity which is very common in spirochætal infections, namely, after apparent recovery spirochætes persist in some part of the host, where for some reason not properly understood they are protected against the antibodies in the blood. Possibly as a result of continual reinforcement of the immune-bodies, these latent infections render the animal resistant against reinfection with the same parasite.

The most familiar example is syphilis, which, before the introduction of organic arsenical compounds for its treatment, was rarely, if ever, completely eradicated. Patients treated with mercury, apparently with success, continued to harbour the spirochæte in the body, generally in the nervous tissue, and such patients were immune against reinfection. With the more complete sterilization obtained by the use of modern methods of treatment, the spirochætes may be completely eliminated from the body and in such cases the host may subsequently lose its immunity and become susceptible to reinfection. It should be noted, however, that certain authorities (e.g. Warthin, 1929) believe that even in these cases the spirochætes are never completely eradicated from the body.

The persistence of similar latent infections has also been demonstrated in the case of animals infected with relapsing-fever spirochætes.

Buschke and Kroó (1923) were the first to show that these organisms may invade the brain and remain there several months after they have disappeared from the blood, and their results have been confirmed repeatedly. Different strains of relapsing fever show this property in very different degrees, depending on the amount of antibodies produced. When mice are inoculated with strains recently isolated from human cases, only a slight immunity reaction is produced and consequently there is not a sufficiently high concentration of immune-bodies in the blood to kill the spirochætes in the brain. Strains which have been kept in mice for long periods cause the development of a higher concentration of immune-bodies and animals infected with such strains show a correspondingly lower proportion of brain infections.

Leptospiral infections also show the same persistence of spirochætes in certain parts of the body, but in these diseases the kidney is the usual site of any latent infection. Wild rats are commonly infected with *S. (Leptospira) icterohæmorrhagiæ*, which, although producing no signs of disease, may be recovered by examination of their kidneys. Such latent infections may persist for several months.

Although not strictly belonging to the group of spirochætes, the *Spirillum* of Sodoku, or rat-bite fever (see Chapter VIII), shows many

points of similarity, and therefore reference may be made to an interesting example of the persistence of latent infections in this disease. Rabbits inoculated with the parasite soon recovered and their blood contained antibodies which destroyed the *Spirilla* in dilutions of 1 in 50 to 1 in 100. Even a year after infection antibodies were still present and also *Spirilla* could be found in the skin and lymphatic glands. Mooser (1927), who made these observations, assumes that the antibodies are unable to pass through the walls of the blood-vessels and that an acquired immunity is kept up by the presence of a latent infection. When the rabbits were sterilized by continued injections of organic arsenicals, the antibodies gradually disappeared and the animals became susceptible to a second infection.

Immunity after recovery.

The immunity problem in connection with the *pallida* group of spirochætes will be discussed in Chapter VII.

Spirochætal infections, in general, are accompanied by the development of immune-bodies in the blood, but very conflicting results have been recorded by different observers regarding the nature and especially the duration of this immunity. As a rule, the immunity following recovery from infection is not of very long duration, especially in the case of relapsing fevers. Leptospiral infections seem to produce a more lasting immunity, for after an attack of spirochætal jaundice the blood of recovered patients has been found to contain antibodies for several years. It is a question, however, whether such persistent immunity may not be the result of latent infections.

The Properties of Immune Serum.

The properties of immune serum have been studied mainly in the case of syphilis, relapsing fevers and infective jaundice, and have been largely used for the identification of various races. The reactions which may be studied are as follows: agglutination, adhesion phenomena due to the presence of thrombocytobarin, Pfeiffer's phenomenon, protection conferred by antisera, fixation of complement, and active immunity in hyperimmunized animals.

In general, the antibodies are very specific, to such a degree that even *passage* through a different host is sufficient to alter the serological properties of any particular strain. For example, Sagel (1928) found that when patients were infected by the bites of ticks infected with *S. berbera* (Algerian relapsing fever), they developed a specific immunity against the same method of infection. These patients, however, were not immune against the same strain after passage through mice and were reinfected by the inoculation of the blood of mice containing this parasite. These results show that far-reaching biological changes are induced by changes in the environment of spirochætes, which may help to explain many of the discordant results of immunity experiments. When any particular spirochæte may produce different strains after changes in passage, it is

not unreasonable to assume that when different strains are maintained under identical conditions for long periods, they may come to resemble each other. The results of observations on relapsing fever also demonstrate the extreme specificity of the serological response, for the spirochætes of the original attack are found to be immunologically distinct from those of the first relapse, and according to some observers, those of each succeeding relapse can also be distinguished.

It would seem, therefore, that too great reliance cannot be placed on these immunological reactions, which at present constitute almost the only method of distinguishing most of the different races of spirochætes.

Agglutination.

This reaction may be used for the sero-diagnosis of spirochætes, as the serum of patients agglutinates the homologous organism in considerable dilutions. It is necessary, of course, to be in possession of the spirochætes on which it is required to make the test, and as their culture, except in the case of leptospira, involves technical difficulties, this method is rarely employed for diagnosis except with patients suspected of being infected with spirochætal jaundice. The process can be observed very readily in the blood of rats or mice infected with relapsing fever, as each crisis is accompanied by the agglutination of the spirochætes preparatory to their disappearance from the blood.

Adhesion phenomenon. (Thrombocytobarin.) (Figs. 26 and 27.)

This reaction, which is more easily observed than the previous one, was reintroduced by Rieckenberg (1917) and provides a ready method of distinguishing different races of spirochætes. It depends on the fact that under the influence of specific antisera the spirochætes are altered in such a way that minute particles, such as gamboge, blood-platelets, or bacteria, adhere to them. Specific antisera can be prepared by inoculating the spirochætes into suitable laboratory animals, such as rabbits, guinea-pigs, rats or mice. The antibody in such sera seems to possess a high degree of thermo-stability (Kritschewski and Tscherikower, 1926). Davis and Brown (1927), who were mainly responsible for introducing the use of this test for the identification of spirochætes, found that it could withstand 65° C. for 2 hours.

The test is made by mixing equal quantities of fresh antiserum with a culture fluid containing the spirochætes. A thin saline suspension of *B. coli*, or any other convenient bacterium, is then added and the mixture incubated at 30° C. for 20 minutes. The liquid is then examined by dark-field illumination and a positive reaction is indicated by the adhesion of bacteria to any of the spirochætes (Brown and Davis, 1927).

Specific antisera may be preserved in the laboratory, but in such cases they must be reactivated with fresh normal guinea-pig serum before being used.

Active-immunity and cross-immunity tests.

The immunity following recovery from spirochætal infections has been largely used for the distinction of the different races of relapsing fever. As previously indicated, its value is questionable, owing to the ease with which strains become modified in their serological properties.

One method of applying this test is to cultivate the spirochæte in the presence of immune serum from various strains. No growth takes place in the tubes containing immune serum of the homologous strain. Another common method of using the cross-immunity test for the comparison of two races of spirochætes is to inoculate each of them into susceptible hosts. After recovery each will be found to have developed a specific active immunity against the race of spirochæte that produced the infection, and reinoculation with this race is without effect. The inoculation of a different race, however, is followed by infection.

The fact that the spirochætes of different relapses of the same infection each produce specific antibodies shows that too great reliance cannot be placed on this test. For example, a mouse was infected with a strain of *S. duttoni* and subinoculations made into other mice with spirochætes of the original attack and of each succeeding relapse, respectively. These mice after recovery were reinoculated with spirochætes from each of the various attacks and were found to be completely immunized only against the type that caused the original infection, but only partially immunized against spirochætes collected at other stages of the disease. Thus a mouse that had been originally infected by the inoculation of second relapse spirochætes could not be reinfected by a subsequent inoculation of other second relapse spirochætes, but showed infections of varying severity if inoculated with organisms of the first attack, or of any other relapses except the second. Similarly, a mouse inoculated with spirochætes from the original attack was immune against the same type, but could be reinfected by the inoculation of spirochætes taken at any of the relapses. Gori (1928), explains these results by assuming that the spirochætes acquire new immunological properties after each relapse.

Cunningham (1925), however, found that there was an alternation of serological types in monkeys infected with the spirochætes of Madras relapsing fever. Thus the organisms of the first attack were identical with those of the second relapse, and the first relapse spirochætes gave the same reactions as the third relapse. Levaditi and Roché (1907), Jancsó (1918), Brussin (1925) and others have studied this phenomenon, and in particular Meleney (1928), who finds that the sequence of strains in relapses is a much more complicated question than originally supposed, for, working with grey squirrels infected with a Chinese strain of relapsing fever, he was able to isolate six strains of spirochætes which retained their specific serological characteristics. Certain relationships were observed in these strains, however, and with one exception these were always between those which appeared, or might have appeared, in alternate attacks.

In principle, the results agree with the view that when strain A is inoculated, strain B appears in the relapse, and when the latter is inoculated strain A appears in the relapse, but with later relapses there was a widening zone of antibody formation, and it was found possible by reinoculation to infect squirrels with four different strains in succession.

Passive Immunity. Serum Therapy.

Although recovery from spirochætal infections is followed by the development of an active immunity, the inoculation of serum containing these antibodies into normal animals has very little protective action. Many attempts have been made to prepare anti-spirochætal sera, but the only one of any use is that obtained by inoculating the horse at frequent intervals for several months with large doses of *S. (Leptospira) icterohæmorrhagiæ*. The resulting serum has been found to give good results in the treatment of infectious jaundice (e.g. Inada, 1917; Pettit, 1928).

Vaccination.

In certain cases the inoculation of dead spirochætes is followed by protection against the same organism. Marchoux and Salimbeni (1903), Levaditi, and Aragão in this way have succeeded in immunizing fowls against infection with *S. anserina*. The inoculation of small doses of living spirochætes may also be used for vaccination, as in the case of this same spirochæte in birds, or *S. (Leptospira) icterohæmorrhagiæ* in the guinea-pig. Various attempts have been made with *Spirochæta pallida*, but hitherto the results have been uncertain.

Toxins.

The manner in which pathogenic spirochætes act on the host is very difficult to explain, for hitherto there is no satisfactory evidence that their action is due to toxins. A guinea-pig may be inoculated with large doses of the filtrate from cultures of *S. (Leptospira) icterohæmorrhagiæ* without showing any ill effects (Pettit, 1928). On the other hand, Schilling and others (1912) record that mice injected with dead *S. recurrentis* died on an average four days later.

Chemotherapy.

The treatment of spirochætosis has been very extensively studied and is the subject of innumerable publications, especially in the case of syphilis, and only a brief reference can be made to it. At the present time the principal remedies in use are mercury, bismuth, arsenic and antimony, and especially the organic salts of arsenic.

Excluding syphilis, which is dealt with in Chapter VII, the spirochætoses at present are treated mainly by the use of organic arsenicals which belong to two groups, one containing pentavalent arsenic, such as atoxyl, stovarsol, &c., and the other trivalent arsenic, of which salvarsan and its derivatives are the best known. All these compounds have little, if any, action on the spirochætes *in vitro*, but *in vivo* are generally found to be

spirochæticidal, though different results are obtained with different infections. The relapsing fevers, as a rule, respond to treatment with salvarsan and its derivatives, but some strains are found to be resistant. Moreover, although spirochætes may disappear from the circulation after treatment, they are often still present in the brain, where the concentration of the therapeutic agent in the blood is not sufficient to kill off all the parasites (Buschke and Kroó, 1923). When an attack of relapsing fever is checked by chemotherapeutic agents the development of antibodies against the infection is arrested at the stage already reached in the body of the host, consequently varying degrees of immunity will be shown by treated persons, depending on the period of the disease when treatment was begun. According to Sagel (1928) the best results in the treatment of relapsing fever are obtained by administering the drug after one or two febrile attacks have resulted in the development of some degree of immunity, also the temperature should be rising and spirochætes present in the circulation. Other observers recommend the injection of a massive dose at the beginning of the infection, for if small doses are employed the spirochætes in the brain remain alive and may become arsenic-resistant.

The most efficient of the many arsenical compounds in use at present seems to be Albert 102, which according to the results of animal experiments shows the most favourable ratio between the curative and the maximum tolerated dose (Hofmann, 1927).

Spirochætes exposed to the action of arsenical drugs may acquire resistance in the same way as trypanosomes. This resistance seems to persist indefinitely, and many of the strains of *S. duttoni* at present in European laboratories are arsenic-resistant. A strain at Hamburg which had been kept for twelve years in laboratory animals was found to have lost its virulence for man and also was resistant to salvarsan. Two months later, after passage through the intermediate host, *Ornithodoros moubata*, the spirochætes had recovered their virulence for man, but were still arsenic-resistant (Plaut, 1925). It is evident, therefore, that care should be exercised in the treatment of relapsing fevers, as the ease with which drug-resistance may be produced might result in such strains becoming widespread in nature.

Intramuscular injections of sodium potassium bismuth tartrate are said to give good results in the treatment of *S. duttoni* infections and to be preferable to novarsenobenzol, especially in the treatment of arsenic-resistant strains (Todd, 1930).

In addition, various gold salts have been tested, and the results of experiments suggest that they may be of great value in the treatment of relapsing fevers. Sanocrysin in doses of 0·0005 to 0·001 gm. was found by Krantz (1926) to cure mice infected with *S. recurrentis*, and if administered at an early stage cut short the attack and prevented relapses.

Solganal, an aromatic organic gold compound, and 'A 69', an adaptation of the same compound for intramuscular use, have been recommended by Steiner and Fischl (1929), who found that in mice infected with

S. recurrentis and *S. duttoni* respectively, both gold compounds had a much higher therapeutic index than novarsenobenzene. In addition, residual brain infections were entirely eliminated.

The leptospiral diseases do not seem to respond to any of the known therapeutic agents, with the exception of bismuth, and in these cases treatment with immune serum is the method which gives most satisfactory results. Recently, however, Sazerac, Nakamura and Kitchevatz (1927) have found that tartrobismuthate of soda will not only protect guinea-pigs against infection with *S. icterohæmorrhagiæ*, but will bring about a cure if given before the fourth day of the disease.

Uhlenhuth and Seiffert (1928 and 1929) have also succeeded in curing infected guinea-pigs by the use of 'Bismuth Yatren A', but it is necessary to administer the drug at the right stage of the disease, which in this case was the sixth or seventh day of infection. When an infected guinea-pig is injected with this bismuth compound, leptospira disappear from the blood but are still present in the internal organs. These organisms are resistant to bismuth, as proved by subinoculation. Later the leptospira disappear entirely from the body and such an animal is immune, not only against reinfection with the homologous strain but also against other varieties.

The mouth spirochætes and bronchial spirochætosis are said to respond to injections of salvarsan or its derivatives, and the intestinal forms respond either to stovarsol taken by mouth or to bismuth compounds.

The action of all chemotherapeutic agents of use in the treatment of spirochætal infections, seems to be mainly indirect, the drug acting through the medium of the natural defence mechanism of the body. Kritschewski and his colleagues (1927¹ & ², 1928) consider that an intact reticulo-endothelial system is necessary for the full action of chemotherapeutic agents in relapsing fever. Although blocking of this system had little effect, splenectomy increased the mortality, the conclusion being that the protective function of the reticulo-endothelial system depends not on phagocytosis of the spirochætes by the reticulo-endothelial cells, but on the production of lytic antibodies. Feldt and Eisenmenger (1928) were unable to confirm these results.

Levaditi, Lépine and Howard (1929) suggest that immune-bodies interact in some way with the drug in killing the spirochætes, since the dose of novarsenobenzene necessary to effect a cure was found to be greater at the beginning of the disease when the parasites are few, than immediately before the crisis when the spirochætes swarm in the blood. This view is also supported by the work of Sagel (1928), mentioned above.

Free-living Spirochætes.

The water leptospira *Spirochæta biflexa*, described in Chapter IX, is by far the commonest free-living spirochæte, but in addition many other forms have been described from fresh and sea water, ranging from such minute

organisms as *Spirochæta minima* Dobell, with a length of only 2 to 2.5μ , up to *S. plicatilis* Ehrenberg, which may reach a length of 500μ , with a diameter of 0.5 to 0.75μ . *Spirochæta gigantea* (Warming, 1875) is said to be 3μ in diameter, but its nature is so doubtful that in the absence of further information as to its structure it cannot be included in this group.

All these free-living forms are found mainly in water containing plenty of organic matter, especially in the presence of sulphuretted hydrogen, and are commonly found growing in association with *Oscillatoria*, *Beggiotoa* and similar organisms. The addition of a small quantity of fæces, or similar organic matter, to river or sea water favours the growth of many of these spirochætes, and in this way various kinds of free-living forms may be found in water that is negative to ordinary examination. Using this method in Thames water the author has found *S. pseudo-pallida* Zuelzer, and *S. pseudo-recurrentis* Zuelzer, which morphologically resemble *S. pallida* and *S. recurrentis* respectively, in addition to a variety of other free-living forms.

Hay infusions sometimes contain an abundant growth of spirochætes, including *S. graminea* Zuelzer, a long slender form reaching 100μ in length and 0.25μ in diameter, which, like *Spirochæta cytophaga*, is able to digest cellulose.

Excluding the water leptospira, the free-living form which has received the most attention is *Spirochæta plicatilis* (Figs. 1 and 2), discovered and named by Ehrenberg in 1838. Although so well known in the literature, this organism seems to be very uncommon, as it has only rarely been found by students of the group. *S. plicatilis* is able to live in both fresh water and the sea, and also in brackish water containing large quantities of decomposing organic matter. It seems to grow best in water containing sulphur bacteria, which liberate sulphuretted hydrogen. This organism has an average length of 100 to 200μ and a thickness of 0.5 to 0.75μ . It is extremely active and flexible during life, showing creeping, coiling and undulating movements of the whole body in addition to the active spiral movements; flagella are absent. In structure it is found to consist of a definite rod-like elastic axial filament supporting the softer protoplasm which is wound round it in the form of a spiral thread. The latter is round in cross-section and the axial filament appears as a dot of denser material. The cytoplasm is alveolar in structure and contains volutin granules, which are generally spaced at regular intervals along the length of the body, one to each spiral turn. Division is transverse, and according to Zuelzer (1910) a slight thickening of the axial filament takes place at the point of division. This thickened portion divides into two dark-staining granules which move apart, and the body of the spirochæte separates between them. In addition to this simple transverse division, as in the case of some other spirochætes, multiple division sometimes occurs, the spirochætes dividing simultaneously into 5 to 15 daughter-individuals each with an average length of 11μ and composed of four spiral turns.

Commensal Spirochætes from Plants and Animals.

A very large number of spirochætes living as commensals have been recorded from various animals and even plants, and it is only possible to refer briefly to some of these forms.

A spirochæte 6 to 8.5μ in length with a thickness of 0.5μ and 3 to 5 spiral turns and pointed extremities has been discovered in the latex of *Euphorbia peplus* in the neighbourhood of Syracuse by Laveran and Franchini (1921), and in addition various spirochætes have been recorded from the alimentary canal of plant-bugs which feed on the latex of Euphorbias and are supposed to derive their infection from the plants. These spirochætal infections in the latex of Euphorbias occur in the same species of plants that are subject to infection with flagellates, and presumably are transmitted by plant-bugs.

With reference to animals, spirochætes have even been recorded from a protozoon, as Fauré-Frémiet (1909) found them living commensally in a ciliate, *Trichodinopsis paradoxa*, parasitic in the intestine of *Cyclostoma elegans*. *Cristispira polydora* Mesnil and Caullery (1916) occurs in the intestine of a marine annelid worm, *Polydora flava*, and a similar form, *Cristispira helgolandica*, has been recorded by Collier (1921) from an Echinoderm, *Asterias rubens*. Molluscs are the hosts of a large number of species belonging to the genus *Cristispira*, which are generally found living in relation with the crystalline style of lamellibranchs (Figs. 3 to 6). The best-known member of this group is *Cristispira balbianii*, which lives in oysters and was described nearly 50 years ago by Certes (1882). The well-developed undulating membrane present in this species led to its being described as a trypanosome, and Perrin (1906) gave an elaborate account of it, based on the supposition that it belonged to the flagellates. The fresh-water mussel often contains *Cristispira anodontæ*, and similar forms can be found without much difficulty by dissecting out the crystalline styles of any lamellibranchs.

The arthropods harbour a number of spirochætes, which inhabit the alimentary canal. When the arthropod feeds by sucking blood, any organisms living in its intestine may have been derived from the vertebrate host, and some spirochætes that have been recorded from blood-sucking insects and ticks are certainly vertebrate parasites. Very few observations have been made on these spirochætes except on their occurrence and dimensions. Some of the animals from which they have been recorded are as follows :

SPECIES.	HOST.	HABITAT.	AUTHORITY.
IXODOIDEA.			
<i>Spirochæta</i> sp. ..	<i>Dermacentor venustus</i>	Cells of oviducts	Becker, 1926.
APHANIPTERA.			
<i>S. ctenocephali</i> ..	<i>Ctenocephalus felis</i> ..	Intestine of larva.	Patton, 1912.

SPECIES.	HOST.	HABITAT.	AUTHORITY.	
HEMIPTERA.				
<i>Spirochæta</i> sp. ..	<i>Lygæus pratensis</i> ..	Intestine ..	Laveran and Franchini, 1920.	
<i>Spirochæta</i> sp. ..	<i>Anthocoris sylvestris</i> ..			
<i>Spirochæta</i> sp. ..	<i>Nysius</i> sp. ..			
ORTHOPTERA.				
<i>Treponema stylopygæ</i> ..	<i>Stylopyga orientalis</i> ..	Intestine ..	Dobell (1912).	
<i>T. parvum</i> ..			Laveran and Franchini (1920).	
<i>Spirochæta periplanetæ</i>				
DIPTERA.				
<i>Spirochæta culicis</i> ..	<i>Culex</i> sp. ..	Intestine and Malpighian tubules.	Lingard and Jennings (see Lingard, 1907).	
		Salivary glands	Jaffé (1907).	
			Ed. and Et. Sergeant (1906 and 1912).	
	<i>Anopheles maculipennis</i>	Midgut of larva	Ed. and Et. Sergeant (1906 and 1912).	
	<i>Theobaldia spathipalpis</i>	Midgut of larva	Ed. and Et. Sergeant (1907).	
	<i>Aedes ægypti</i> ..	Gut of larva and adult.	Noc and Stévenel (1913).	
		Malpighian tubules.	Noc (1920).	
<i>Spirochæta phlebotomi</i> .	<i>Phlebotomus perniciosus</i>		Pringault (1921).	
<i>Spirochæta</i> sp. ..	<i>Chironomus plumosus</i>	Intestine ..	L. Léger (1902).	
<i>Spirochæta</i> sp. ..	<i>Drosophila confusa</i> ..	Intestine of larva pupa and adult.	Chatton (1912).	
<i>Spirochæta noelleri</i>	<i>Simulium noelleri</i> ..	Intestine of larva.	Zuelzer (1925).	
<i>Sp. glossinæ</i> ..	<i>Glossina palpalis</i> ..	Stomach ..	Novy and Knapp (1906 ²).	
<i>Sp. glossinæ</i> ..	<i>G. brevipalpis</i> ..	—	Keysselitz and Mayer (1908).	
	<i>G. tachinoides</i> ..	—	Macfie (1914).	
<i>Spirochæta melophagi</i> .	<i>Melophagus ovinus</i> ..	Intestine, ovaries and pupa.	Porter (1910).	
<i>Spirochæta</i> sp. ..	<i>Ptychoptera contaminata</i> .	Intestine of larva.	Léger and Duboscq (1909).	
NEUROPTERA.				
<i>S. termitis</i> ..	<i>Termes flavipes</i> ..	Intestine of workers.	Leidy (1881).	
<i>S. minei</i> ..			<i>T. lucifugus</i> ..	Dobell (1910).
			<i>Calotermes militaris</i> ..	Prowazek (1910).
	<i>C. flavicollis</i> ..			
<i>S. leucotermis</i> ..	<i>T. lucifugus</i> ..		Hollande (1922).	
<i>S. grassi</i> ..	Termites ..	Intestine ..	Doflein (1911).	

SPECIES.	HOST.	HABITAT.	AUTHORITY.
NEUROPTERA—continued.			
<i>Spirochæta</i> sp. ..	<i>Cryptotermes brevis</i> .. <i>Kalotermes schwarzi</i> and many other American termites.	Intestine ..	Damon (1926).
TRICHOPTERA.			
<i>Spirochæta</i> sp. ..	<i>Limnophilus</i> , &c. ..	Intestine of larvæ, occa- sionally in pupa, never in adult.	Mackinnon (1910).

The tunicata are also the host of two species of spirochætes, *Spirochæta cæsire septentrionalis* and *S. cæsire retortiformis*, probably belonging to the genus *Cristispira*, which were described by Hellman (1913) from the excretion organs of *Cæsira* (*Molgula*) living in the White Sea.

The intestinal tract of every class of vertebrates may contain spirochætes, which as a general rule seem to be living as harmless commensals. The main forms occurring in man are discussed on p. 141, but in addition similar varieties may be found in many mammals. A list of the species of animals from which spirochætes have been recorded is given by Pettit (1928).

Genera allied to Spirochætes.

In addition, reference should be made to certain forms showing various degrees of affinity with the typical spirochætes, and suggesting possible lines of development.

Heliconema Sanarelli, 1927.

The genus *Heliconema* was founded by Sanarelli (1927) to include *Heliconema vincenti*, a cæcal spirochæte of guinea-pigs which he isolated in pure culture. This parasite showed all stages between spirochætes and fusiform bacilli, and the name was suggested to include such polymorphic spirochætes.

An abundant growth of this organism can be obtained on agar slopes containing a piece of fresh guinea-pig tissue in each. Large doses inoculated into guinea-pigs cause death, and the spirochæte can be recovered from the heart-blood or organs. In old cultures coccus forms are developed in large numbers.

It was found that growth in ordinary broth is favoured by the presence of *B. mesentericus vulgaris* or the admixture of a sterile filtrate of a culture of this bacillus. The latter substance also produces changes in form, many of the spirochætes changing into fusiform bacilli. They lose their power of rotation and straighten out into filaments or bands which are twisted at intervals, and in stained films give the appearance of a number of fusiform elements attached end to end. When division takes place at these twisted points the fusiform bacilli are produced. The serum of guinea-pigs immunized against the cæcal spirochæte is found to agglutinate

both the spirochaetal form and also the fusiform bacilli. Also when guinea-pigs are inoculated with fusiform bacilli in sufficient doses, death is produced and both spirochætes and fusiform bacilli can be recovered from the organs. Further observations on this interesting organism are much to be desired.

Cristispirella Hollande, 1921.

This genus was founded by Hollande (1921) to include a spiral organism occurring in the small intestine of the guinea-pig and named by him *Cristispirella caviæ*. This parasite consists of a fine axial filament, describing two or three wide spirals. Along the edge of the filament is developed a wide undulating membrane, recalling that in *Cristispira*. The body of the parasite measures 9 to 12 μ in length and 1.5 to 2 μ in breadth, and the extremities are pointed. It differs from the genus *Cristispira* in the absence of a chambered structure and in being much smaller. Multiplication is by transverse division, preparatory to which there is a thickening of the chromatic part of the organism and two granules appear, one on each side of the point of division. In addition, encystment is said to occur, the parasite coiling itself up and secreting a substance which forms a cyst about 1.5 to 2 μ in diameter.

A form discovered by Mesnil and Caullery (1916) in the intestine of a marine annelid worm, *Polydora flava*, and named by them *Cristispira polydoræ*, is considered by Hollande to belong to this genus.

Spirella Duboscq and Lebailly, 1912¹.

This genus was founded to include *Spirella regaudi*, an organism occurring in the stomach of various carnivora, especially dogs and cats, the great majority of which are infected. The organism is a flexible spiral terminating in a flagellum at each extremity. Considerable variations in size may be observed, from fine filamentous forms, with a thickness of 0.2 μ , up to thick forms with a diameter of 0.4 μ , tapering to 0.25 μ at either extremity. The slender forms have an average length of 3 to 4 μ and the thicker forms an average of about 6.0 μ . The dimensions of each spiral turn varies in the two forms, the former being 0.5 μ in length and 0.5 μ in diameter, whilst the latter are 1.0 μ in length and 1.2 μ in diameter. Consequently the slender forms generally have 6 to 8 spiral turns, and the thicker forms 5 or 6 turns. All intermediate stages may be observed between the thin forms and the thick ones, and the number of spiral turns may vary from 2 or 3 up to as many as 20. The flagellum is 3 to 4 μ in length. Multiplication is by transverse division.

This interesting organism occurs in the gastric epithelium, mainly associated with the oxyntic cells, and is also found in the thick mucus lining the stomach. It seems to be almost confined to this region of the body, never having been found in the duodenum and only rarely in the œsophagus. It is very common in dogs, cats, monkeys, &c., and white rats may be infected by feeding them with infected material (Kasai and Kobayashi, 1919).

Spirella seems to occupy an intermediate position between spirochætes and *Spirilla*, in its general form resembling the latter, but in the flexibility of its body recalling the spirochætes.

Paraspirillum Dobell, 1911. (Figs. 15 to 17.)

This genus was founded by Dobell (1911) for the inclusion of a remarkable organism, *P. vej dovskii*, found in a culture of fresh-water Cyanophyceæ obtained from the River Granta above Cambridge. The organism consists of spiral or S-shaped rods varying from short individuals 8μ in length, up to a maximum of 25μ , the average length being about 15μ . The body shows a thickening about the middle, where the diameter is 1.5 to 2μ , and tapers towards each extremity. A definite nucleus can be seen lying in the middle of the organism. Some of the forms show a terminal flagellum at one or both extremities (Fig. 16), but its presence is not constant. Multiplication is by transverse division, the nucleus dividing amitotically (Fig. 17).

A small *Spirillum* often present in the hind gut of the common cockroach is considered by Dobell to have a nucleus resembling that of *Paraspirillum vej dovskii*, though on a smaller scale.

‘*Spirochæta*’ *cytophaga* *Hutchinson and Clayton, 1919.*

‘*Spirochæta*’ *cytophaga* lives in the soil, and has the peculiarity of breaking down cellulose with comparative ease. It may be cultured in an agar medium containing mineral salts, sodium nitrate and chemically pure cellulose. The most satisfactory results were obtained with 1.5 per cent. agar and 0.25 per cent. sodium nitrate dissolved in a stock solution consisting of KH_2PO_4 , 1 gm.; CaCl_2 , 0.1 gm.; $\text{MgSO}_4 + 7\text{H}_2\text{O}$, 0.3 gm.; NaCl , 0.1 gm.; Fe_2Cl_6 , 0.01 gm.; and H_2O , 1,000 c.cm. After preparing the agar, 1 per cent. of finely ground filter-paper is added and the mixture tubed or plated, and finally sterilized in the autoclave.

Cultures of *Spirochæta cytophaga*, after 8 to 10 days’ incubation at 30°C ., show the presence of two types of colony, one composed almost entirely of filamentous forms, whilst in the other type a large coccus or sporoid form occupies a predominant position. All efforts to separate these two forms were unsuccessful, and in addition stages were observed in the germination of the sporoid or coccus form into the flexible filamentous stage. The latter are feebly motile and consist of undulating forms about 3μ long and 0.3 to 0.4μ in diameter with tapering extremities. In addition, long twisted filaments are sometimes formed which may reach a length of 40μ . The filamentous forms multiply by transverse division, but in older cultures sporoid forms are developed. These are spherical or ovoid cells approximately 1.5μ in diameter which may contain one or two chromatic granules.

The affinities of this organism are somewhat obscure, but the sporoids closely resemble coccus forms occurring in association with *Spirella regaudi*. In both instances it is difficult in stained preparations to decide whether these sporoid or coccus forms are the result of degeneration or

represent a stage in development. Hutchinson and Clayton (1919) were definitely of the opinion that in the case of *S. cytophaga* these sporoids could develop into the spiral form, and Dr. A. C. Stevenson informs me that as a result of studying *Spirella regaudi* in the stomach of dogs and cats he is convinced that a similar development takes place in the case of this organism. The appearance of the filamentous stage of *S. cytophaga* recalls that of *Paraspirillum*, and it is obvious that it does not belong to the genus *Spirochæta*.

Spirochætes of Doubtful Significance in Human Disease.

A very large number of spirochætes have been recorded from various parts of the human body, and in many instances it has been suggested that these various organisms are of pathogenic significance in a variety of diseases the ætiology of which is still unknown. It is unnecessary to give details of all these various records, most of which have died a natural death, but it cannot be too strongly emphasized that the mere presence of a spirochæte in diseased tissue in itself is of no more significance than that of any other of the many bacteria commonly present in such sites. Moreover, the filamentous forms assumed by degenerating blood-cells often resemble spirochætes to a most extraordinary degree, and have frequently been described as these organisms by observers unfamiliar with their appearance. Also, in films stained by silver impregnation methods, artefacts are sometimes produced which may easily be mistaken for spirochætes. It is a good rule in stained preparations not to accept any spiral organism as a spirochæte unless both extremities are quite distinct. Moreover, when examining living spirochætes under the dark-ground illumination, it is necessary to see the whole organism quite distinct, and it should show definite spiral and translatory movements. Degenerating cells often give off filaments which may rotate and undulate in the surrounding medium, but such pseudo-spirochætes show no definite translatory movements, and generally one or both of the extremities are ill-defined.

Spirochæta argentinensis Kuhn and Steiner, 1917.

This spirochæte was originally described by Kuhn and Steiner (1917), who found it in the livers of guinea-pigs and rabbits inoculated with the blood or cerebrospinal fluid of patients suffering from disseminated sclerosis. It was described as being moderately active, somewhat worm-like, seldom rotatory, and having a short straight flagellum at each end, and, according to Adams, Blacklock, Dunlop and Scott (1924), it is 8 to 15 μ in length with 5 to 9 irregular spirals. It is thicker than *S. pallida* and less refringent and mobile. Noguchi (1928), as a result of the examination of Levaditi-stained preparations of Steiner, states that the organism is more like a spirillum than a spirochæte, and resembles *S. minus*, the organism of rat-bite fever.

The causal relationship of *S. argentinensis* to disseminated sclerosis is still very doubtful in spite of several observers having recorded its presence in animals inoculated with material from human patients.

W. E. Bullock (now Gye), in 1913, was the first to show that the inoculation of cerebrospinal fluid from these cases into rabbits produced paralysis (see Gye, 1921), and these results were subsequently confirmed by Kuhn and Steiner (1917 and 1920), who were able to obtain passages of the disease in rabbits and guinea-pigs by the inoculation of blood. Although the spirochæte to which the above name was given, was present in the blood of these animals, it was not found in human cases of the disease. Marinesco (1919) also found similar spirochætes in guinea-pigs inoculated with the disease.

Siemerling (1918) was the first to record the presence of spirochætes in the brain of a patient who died of disseminated sclerosis, and similar results were obtained by Speer (1921), but the latter was unable to find the organisms in stained sections of the brain, although they were seen in the cerebrospinal fluid during life. A. Pettit (1928) succeeded in finding spirochætes in a monkey and young rabbits inoculated from four cases of the disease and obtained passages in two instances. Adams, Blacklock and M'Cluskie (1925) found spirochætes distinct from *S. cuniculi* in the brains of rhesus monkeys and rabbits inoculated with material from cases of disseminated sclerosis.

Gye (1921), in an experimental investigation of 21 cases of the disease, including the inoculation of 129 rabbits and 15 guinea-pigs, obtained positive results, involving paralysis of the hind legs, in 17 rabbits and 9 guinea-pigs inoculated with cerebrospinal fluid from 7 cases of the disease. Passages were successful in 3 cases, and occasionally the death of the animal was produced. No marked lesions were present and spirochætes could never be isolated.

Similar negative results have been recorded by a number of observers and recently evidence has been brought forward in support of the view that the causal agent of disseminated sclerosis is an organism similar to that occurring in bovine pleuro-pneumonia (Chevassut, 1930).

These observations are definitely opposed to the view that *Spirochæta argentinensis* is the causal agent, and it is probable that this organism is merely one of the many saprophytic spirochætes that may occur in animals.

The Spirochæte of Mumps.

It had previously been shown by Nicolle and Conseil (1913) that rhesus monkeys may be infected by the inoculation into the parotid gland of the material obtained by centrifuging the contents of the mouth of a human case of the disease. Moreover, Granata (1908) had also been able to infect rabbits, and Wollstein cats, by similar methods. Kermorgant (1925) made cultures from the saliva in a medium consisting of rabbit-serum 20 c.cm., horse red cells 50 c.cm., and 0·8 per cent. NaCl 750 c.cm. The pH was about 6·6 to 6·7 and the cultures were grown under vaseline.

Under these conditions a growth of spirochætes and various bacteria was obtained. The spirochætes were especially abundant in the upper part of the culture, and by adding horse-serum to the medium and making successive transplantations from the top of the culture, the spirochæte and a motile bacillus were isolated.

The spirochæte is said to be 12 to 16 μ in length and 0.3 μ in thickness, resembling *S. pallida*, but is not dissolved by saponin. Multiplication is by transverse division, and in cultures of about two months old, granules are formed. These give rise to spiral forms with only one spiral turn and eventually to the complete spirochæte. A culture of spirochætes and the associated bacillus when filtered through a Chamberland candle, L 2 or L 3, shows only granule forms, but when a pure culture of the bacillus is added to the filtrate and the mixture is incubated the spirochætes reappear and grow together with the bacillus.

This associated bacillus is rod-shaped with rounded ends and has a single long flagellum or cilium; it is Gram-negative and a facultative-aerobe, and is generally present in the mouth. Its presence was found to be essential to the growth of the spirochætes in culture.

The inoculation of these cultures into monkeys is said to have reproduced the disease, in three instances the parotid being affected, and in another case an intratesticular injection in a *Macacus sinicus* produced first a left orchitis, subsequently parotiditis, then a right orchitis, and finally infection of the maxillary and submaxillary. Rabbits were also infected but about half of them were found to be resistant. The virulence of the cultures was found to diminish gradually, but infections were produced up to the fourteenth subculture after an interval of six months from the primary culture. The associated bacillus does not produce any effect when inoculated into animals, but the filtrate of the joint culture, after passing through a L 2 or L 3 candle, is virulent.

The serum of a convalescent agglutinates the spirochæte in dilutions of 1 : 100 and immobilizes them at dilutions of 1 : 1,000.

The examination of sections of infected glands from cases of mumps has never revealed the presence of spirochætes, and therefore if the spirochæte described by Kermorgant is the causal organism, it is necessary to assume that it is present in an unrecognized form such as the granule. The evidence adduced, however, is not very convincing, and it seems possible that the author cultivated one of the many normal inhabitants of the mouth, together with the virus, whatever its nature, responsible for reproducing the symptoms of the disease in experimental animals. At present the view that mumps is of spirochætal origin requires confirmation. (See also Vol. VII, Chapter XXI.)

REFERENCES.

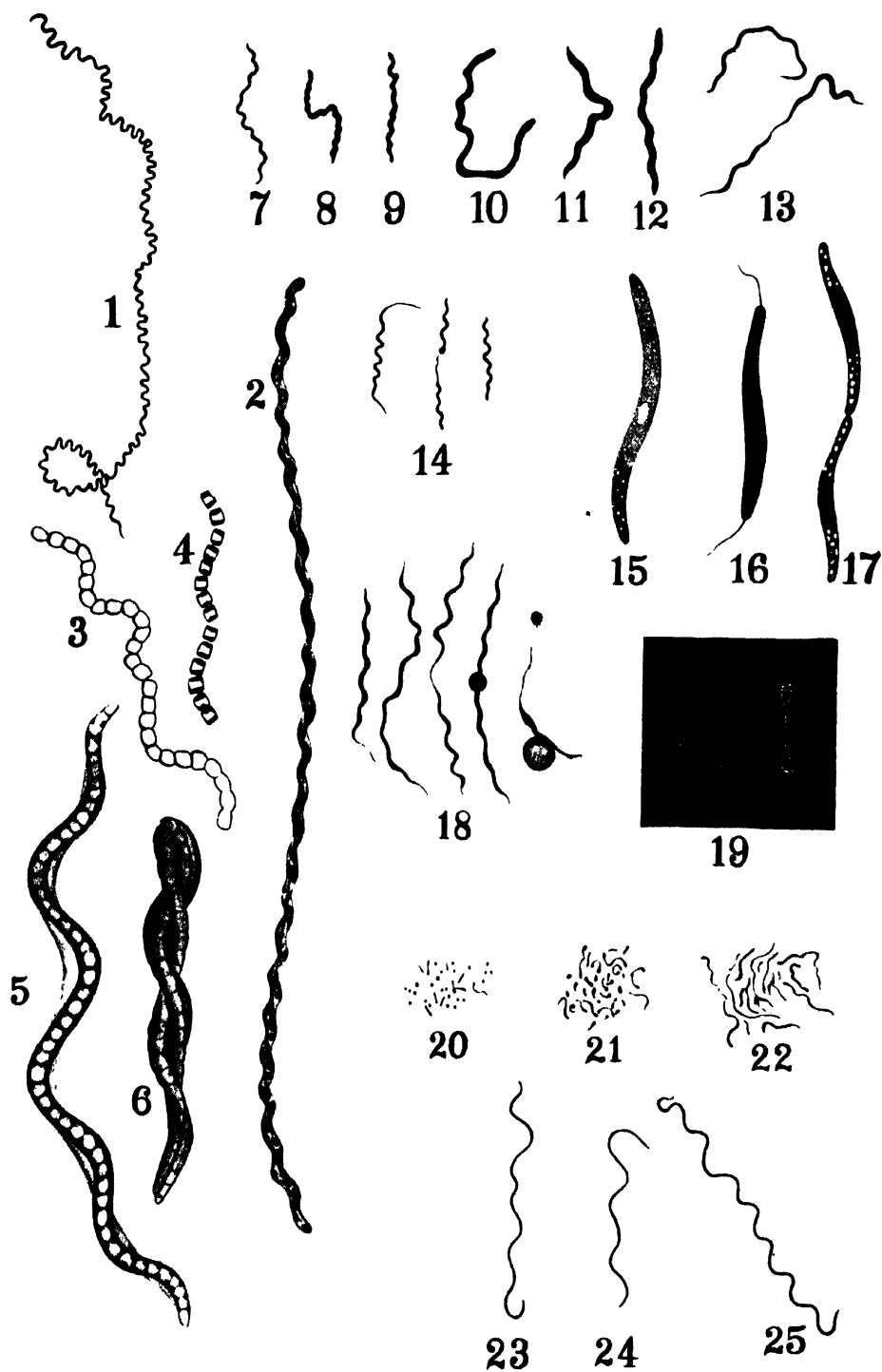
- ACHITOUV, M., 1924, *C.R. Soc. Biol.*, Paris, **91**, 1192.
ADAMS, D. K., BLACKLOCK, J. W. S., DUNLOP, E. M. & SCOTT, W. H., 1924, *Quart. J. Med.*, **17**, 129.
ADAMS, D. K., BLACKLOCK, J. W. S. & M'CLUSKIE, J. A. W., 1925, *J. Path. Bact.*, **28**, 115.

- AKATSU, S., 1927, *J. Exp. Med.*, **25**, 375.
- ANDERSON, T. E., 1928, *C.R. Soc. Biol.*, Paris, **99**, 378.
- BAERMANN, G. & ZUELZER, M., 1928, *Zbl. Bakt.*, Abt. I, Orig., **105**, 345.
- BALFOUR, A., 1911, *J. Trop. Med.*, **14**, 113.
- BECKER, F. E., 1926, *J. Infect. Dis.*, **39**, 81.
- BREINL, A. & KINGHORN, A., 1906, *Mem. Lpool. Sch. Trop. Med.*, **21**, 1.
- BROWN, H. C. & DAVIS, L. J., 1927, *Brit. J. Exp. Path.*, **8**, 397.
- BRUSSIN, A. M., 1925, *Z. ImmunForsch.*, **44**, 328.
- BRUYNOGHE, R. & DUBOIS, A., 1925, *C.R. Soc. Biol.*, Paris, **92**, 466; 1927, *ibid.*, **96**, 1403.
- BULLOCK, W. E. (now GYE), 1913, *Lancet*, Lond., ii, 1185.
- BUSCHKE, A. & KROG, H., 1923, *Deuts. med. Wschr.*, **49**, 1435.
- CANTACUZÈNE, J., 1910, *C.R. Soc. Biol.*, Paris, **68**, 75.
- CERTES, A., 1882, *Bull. Soc. Zool. Fr.*, **7**, 347.
- CHATTON, E., 1912, *C.R. Soc. Biol.*, Paris, **73**, 212.
- CHEVASSUT, K., 1930, *Lancet*, Lond., i, 552.
- COLLIER, 1921, *Zbl. Bakt.*, Abt. I, Orig., **86**, 132.
- CUNNINGHAM, J., 1925, *Trans. Soc. Trop. Med. Hyg.*, Lond., **19**, 11.
- DAMON, S. R., 1926, *J. Bact.*, **11**, 31.
- DAVIS, L. J. & BROWN, H. C., 1927, *Trans. Soc. Trop. Med. Hyg.*, Lond., **21**, 113.
- DELAMARE, G. & ACHITOUV, 1925, *C.R. Soc. Biol.*, Paris, **92**, 81.
- DOBELL, C., 1910, *Spolia zeylan.*, **7**, 81; 1911, *Arch. Protistenk.*, **24**, 97; 1912¹, *Proc. Roy. Soc., B.*, **85**, 186; 1912², *Arch. Protistenk.*, **26**, 151; 1923, *Parasitology*, **15**, 308.
- DOFLEIN, F., 1911, *Probleme der Protistenkunde, II, Die Natur der Spirochäten*, Fischer, Jena.
- DUBOSCQ, O. & LEBAILLY, C., 1912¹, *C.R. Acad. Sci.*, Paris, **154**, 662; 1912², *ibid.*, **154**, 835; 1913, *Arch. Zool. exp. gén.*, **52**, 9.
- DUTTON, J. E. & TODD, J. L., 1907, *Lancet*, Lond., ii, 1523.
- EHRENBERG, C. G., 1834, *Phys. Abh. preuss. Akad. Wiss.*, Berl., p. 145 (Gelesen Juli 1832, gedruckt Mai 1834).
- FANTHAM, H. B., 1911, *Ann. Trop. Med. Parasit.*, **5**, 479.
- FAURÉ-FRÉMIET, 1909, *C.R. Soc. Biol.*, Paris, **67**, 113.
- FELDT, A. & EISENMENGER, C., 1928, *Z. Hyg. InfektK.*, **109**, 410.
- GORI, P., 1928, *Boll. Ist. sieroter.*, Milano, **7**, 1.
- GRANATA, S., 1908, *Med. ital.*, Napoli, **6**, 647.
- GROSS, J., 1912, *Zbl. Bakt.*, Abt. I, Orig., **65**, 83.
- GYE, W. E., 1921, *Brain*, **44**, 213.
- HELLMAN, G., 1913, *Arch. Protistenk.*, **29**, 22.
- HINDLE, E., 1911, *Parasitology*, **4**, 133; 1912¹, *Proc. Camb. Phil. Soc.*, **16**, 457; 1912², *Parasitology*, **4**, 463; 1925, *Brit. Med. J.*, ii, 57.
- HOFMANN, A., 1927, *Arch. Schiffs- u. Tropenhyg.*, **31**, 182.
- HOGUE, M. J., 1922, *J. Exp. Med.*, **36**, 617.
- HOLLANDE, A. C., 1921, *C.R. Acad. Sci.*, Paris, **172**, 1693; 1922, *Arch. Zool. exp. gén.*, **61**, 2.
- HUTCHINSON, H. B. & CLAYTON, J., 1919, *J. Agric. Sci.*, **9**, 143.
- INADA, R., 1917, *Tokyo Med. News*, 1917, 24.
- INADA, R. & IDO, Y., 1915, *Tokyo Ijishinshi*, No. 1908.
- INADA, R., IDO, Y., HOKI, R., ITO, H. & WANI, H., 1916, *J. Exp. Med.*, **24**, 485.
- JAFFÉ, J., 1907, *Arch. Protistenk.*, **9**, 100.
- JANCsó, V., 1918, *Zbl. Bakt.*, Abt. I, Orig., **81**, 457.
- KASAI, K. & KOBAYASHI, R., 1919, *J. Parasit.*, **6**, 1.
- KEYSSELITZ, G. & MAYER, M., 1908, *Arch. Schiffs- u. Tropenhyg.*, **12**, 532.
- KERMORGANT, Y., 1925, *Ann. Inst. Pasteur*, **39**, 565.
- KISSMEYER, A., 1923, *Münch. med. Wschr.*, **70**, 775.
- KLIGLER, I. J. & ROBERTSON, O. H., 1922, *J. Exp. Med.*, **35**, 303.
- KNOWLES, R., GUPTA, B. M. DAS & BASU, B. C., 1929, *Far Eastern Assoc. Trop. Med. Trans. Seventh Congress*, British India, 1927, **2**, 573.
- KRANTZ, W., 1923, *Münch. Med. Wschr.*, **70**, 628; 1926, *Klin. Wschr.*, **5**, 1831.
- KRITSCHIEWSKI, J. L., 1927¹, *Klin. Wschr.*, **6**, 1370; 1927², *Zbl. Bakt.*, Abt. I, Orig., **104**, 214*.

- KRITSCHIEWSKI, J. L. & FRIEDE, K. A., 1925, *Arch. Schiffs- u. Tropenhyg.*, **29**, Beih. 5, [707].
- KRITSCHIEWSKI, J. L. & RUBINSTEIN, P. L., 1927, *Z. ImmunForsch.*, **51**, 27.
- KRITSCHIEWSKI, J. L. & SCHAPIRO, S., 1928, *Z. ImmunForsch.*, **56**, 308.
- KRITSCHIEWSKI, J. L. & TSCHERIKOWER, R. S., 1926, *Z. ImmunForsch.*, **46**, 207.
- KUHN, P. & STEINER, G., 1917, *Med. Klink.*, **13**, 1007; 1920, *Z. Hyg. InfektKr.*, **90**, 417.
- LAPIDARI, M. & SPARROW, H., 1928, *Arch. Inst. Pasteur, Tunis*, **17**, 191.
- LAVERAN, A. & FRANCHINI, G., 1920, *Bull. Soc. Path. exotique*, **13**, 331; 1921, *ibid.*, **14**, 205.
- LÉGER, L., 1902, *C.R. Acad. Sci.*, Paris, **134**, 1317.
- LÉGER, L. & DUBOSQ, O., 1909, *Bull. Acad. Belg. Cl. Sci.*, No. 8, 885.
- LEIDY, J., 1881, *J. Acad. Nat. Sci. Philad.*, 2nd Ser., **8**, 441.
- LEISHMAN, W. B., 1920, *Lancet*, Lond., ii, 1237.
- LEVADITI, C., LÉPINE, P. & HOWARD, A., 1929, *C.R. Soc. Biol.*, Paris, **100**, 1068.
- LEVADITI, C. & ROCHÉ, J., 1907, *C.R. Soc. Biol.*, Paris, **62**, 815.
- LINGARD, A., 1907, *J. Trop. Vet. Sci.*, **2**, 261.
- MACFIE, J. W. SCOTT, 1914, *Ann. Trop. Med. Parasit.*, **8**, 464.
- MACKINNON, D. L., 1910, *Parasitology*, **3**, 245.
- MANOUÉLIAN, Y., 1930, *C.R. Soc. Biol.*, Paris, **104**, 249.
- MARCHOUX, E. & COUVY, L., 1913, *Ann. Inst. Pasteur*, **27**, 450 and 620.
- MARCHOUX, E. & SALIMBENI, A., 1903, *Ann. Inst. Pasteur*, **17**, 569.
- MARINOSCO, G., 1919, *Rev. neurol.*, **26**, 481.
- MARTIN, L. & PETTIT, A., 1919, *Spirochétose Ictérohémmorragique*, Paris.
- MELENEY, H. E., 1928, *J. Exp. Med.*, **48**, 65.
- DE MELLO, F. & DE ANDRADE, M., 1922, *Bull. Soc. Path. exotique*, **15**, 284.
- MESNIL, F., 1921, *Médecine*, **2**, 930.
- MESNIL, F. & CAULLERY, M., 1916, *C.R. Soc. Biol.*, Paris, **79**, 1118.
- MOOSER, H., 1927, *Schweiz. med. Wschr.*, **57**, 1154.
- MORODER, J., 1929, *Arch. Schiffs- u. Tropenhyg.*, **33**, 603.
- MÜHLENS, P. & HARTMANN, M., 1906, *Z. Hyg. InfektKr.*, **55**, 81.
- NAGÃO, M., 1923, *J. Jap. Soc. Vet. Sci.*, **2**, 127.
- NICOLLE, C., 1927, *Arch. Inst. Pasteur Tunis*, **16**, 207.
- NICOLLE, C., BLAIZOT, L. & CONSEIL, E., 1913, *Ann. Inst. Pasteur*, **27**, 204.
- NICOLLE, C. & CONSEIL, E., 1913, *C.R. Acad. Sci.*, Paris, **157**, 340.
- NICOLLE, C. & LEBAILLY, C., 1920, *Arch. Inst. Pasteur Tunis*, **11**, 131.
- NOC, F., 1920, *Bull. Soc. Path. exotique*, **13**, 672.
- NOC, F. & STÉVENEL, L., 1913, *Bull. Soc. Path. exotique*, **6**, 708.
- NOGUCHI, H., 1912, *J. Exp. Med.*, **16**, 199 and 620; 1913, *Pr. méd.*, **21**, 801; 1917, *J. Exp. Med.*, **25**, 755; 1919, *ibid.*, **30**, 26; 1928, *The Spirochetes*, in Jordan and Falk's *The newer knowledge of bacteriology and immunology*, Univ. Chicago Press.
- NOVY, F. G. & KNAPP, R. E., 1906¹, *J. Infect. Dis.*, **3**, 385; 1906², *ibid.*, **3**, 291.
- OBERMEIER, O., 1873, *Zbl. med. Wiss.*, **11**, 145.
- PATTON, W. S., 1912, *Ann. Trop. Med. Parasit.*, **6**, 357.
- PERRIN, W. S., 1906, *Arch. Protistenk.*, **7**, 131.
- PETTIT, A., 1928, *Contribution à l'étude des Spirochétidés*, Vanves.
- PLAUT, F., 1925, *Deuts. med. Wschr.*, **51**, 387.
- PONSELLE, A., 1910, *C.R. Soc. Biol.*, Paris, **69**, 307.
- PORTER, A., 1910, *Quart. J. Micr. Sci.*, **55**, 189.
- PRINGAULT, E., 1921, *C.R. Soc. Biol.*, Paris, **84**, 209.
- V. PROWAZEK, S., 1909, *Mem. Inst. Oswaldo Cruz*, **1**, 79; 1910, *Arch. Schiffs- u. Tropenhyg.*, **14**, 297.
- REITER, H., 1916, *Deuts. med. Wschr.*, **42**, 1282.
- REPACI, G., 1911, *C.R. Soc. Biol.*, Paris, **70**, 784.
- RIECKENBERG, H., 1917, *Z. ImmunForsch.*, Tl. I, Orig., **26**, 53.
- SAGEL, W., 1928, *Arch. Schiffs- u. Tropenhyg.*, **32**, Beih. 6, [283].
- SANARELLI, G., 1927, *Ann. Inst. Pasteur*, **41**, 679.
- SAZERAC, R., NAKAMURA, H. & KITCHEVATZ, M., 1927, *C.R. Acad. Sci.*, Paris, **184**, 411.
- SCHAUDINN, F., 1905, *Deuts. med. Wschr.*, **31**, 1728.

- SCHILLING, CL., VON KROGH, M., SCHRAUTH, W. & SCHOELLER, W., 1912, *Z. Chemother.*, Orig., **1**, 21.
- SEGUIN, P., 1930, *C.R. Soc. Biol.*, Paris, **104**, 247.
- SERGENT, ED. & ET., 1906, *C.R. Soc. Biol.*, Paris, **60**, 291 ; 1907, *Ann. Inst. Pasteur*, **21**, 251 ; 1912, *C.R. Soc. Biol.*, Paris, **73**, 36.
- SERGENT, ED. & FOLEY, H., 1914, *C.R. Acad. Sci.*, Paris, **159**, 119.
- SIEMERLING, E., 1918, *Berl. klin. Wschr.*, **55**, 273.
- SPEER, E., 1921, *Münch. med. Wschr.*, **68**, 425.
- STEINER, G. & FISCHL, V., 1929, *Klin Wschr.*, **8**, 582.
- SWELLENGREBEL, N. H., 1907, *Ann. Inst. Pasteur*, **21**, 448 and 562.
- TODD, J., 1930, *Brit. Med. J.*, **1**, 312.
- TODD, J. L. & WOLBACH, S. B., 1914, *J. Med. Res.*, **30**, 27.
- TUNNICLIFF, R., 1906, *J. Infect. Dis.*, **3**, 148.
- UHLENHUTH, P. & SEIFFERT, W., 1928, *Med. Klinik.*, **24**, 584, 1929, *Zbl. Bakt.*, Abt. I, Orig., **114**, 241.
- UHLENHUTH, P. & ZUELZER, M., 1922, *Klin Wschr.*, **1**, 2124.
- WARMING, 1875, *Videnskabl. Meddel. Naturh. Foren.* Kjöbenhavn.
- WARTHIN, A. S., 1929, *Brit. Med. J.*, ii, 236.
- WENYON, C. M., 1926, *Protozoology*, London, Baillière, Tindall & Cox.
- WOLBACH, S. B. & BINGER, C. A. L., 1914, *J. Med. Res.*, **30**, 23.
- ZUELZER, M., 1910, *Zool. Anz.*, **35**, 795 ; 1925, *Die Spirochäten (Nachtrag)*, in v. PROWAZEK'S *Handbuch der pathogenen Protozoen*, III.
- ZUELZER, M. & PHILIPP, E., 1925, *Strahlentherapie*, **20**, 737, also included in 1926, *Arb. Gesundheitsamt.*, Berl. **56**, 589.

PLATE A.



DESCRIPTION OF PLATE A (Figs. 1 to 25).

Various types of spirochætes.

Fig. 1. *Spirochæta plicatilis*, as seen in the living condition in water. ($\times$ ca. 500).

Fig. 2. Ditto, stained to show the axial filament. ($\times$ ca. 2,000).

Figs. 3 and 4. *Cristispira tapetos*, from the crystalline style of a mussel, showing successive stages in the breaking up of the body into short forms, resembling spores. ($\times$ ca. 2,000).

Figs. 5 and 6. *Cristispira veneris*, from the crystalline style of *Venus casta*, showing (5) an average individual, stained with Giemsa to show the chambered structure and the crista; and (6) a dividing form in the last stage of incurvation. ($\times$ ca. 2,000).

Figs. 7 to 13. Mouth spirochætes. (All $\times$ ca. 2,000).

Figs. 7 to 9. *Spirochæta dentium*, stained with Loeffler's flagellar stain, which exaggerates the thickness.

Figs. 10 to 12. *Spirochæta buccalis*, stained with Loeffler's flagellar stain, also of exaggerated thickness.

Fig. 13. Ditto, wet film, stained with methylene blue. The upper figure represents a normal individual, and the lower the last stage in transverse division.

Fig. 14. *Spirochæta balanitidis*, from a prepucial ulcer, stained with Loeffler's flagellar stain, showing on the left an individual with drawn-out extremities, in the centre a dividing form, and on the right a normal individual. ($\times$ ca. 1,300).

Figs. 15 to 17. *Paraspirillum vej dovskii*, from a culture of fresh-water Cyanophyceæ. ($\times$ ca. 2,000). Fig. 15, drawn from life; Fig. 16, an organism from a wet film, deeply stained with iron-hæmatoxylin and showing flagella; and Fig. 17, a late state in division, also stained with iron-hæmatoxylin, each daughter-individual showing a nucleus and metachromatic granules.

Fig. 18. *Spirochæta recurrentis*, drawn from stained dried blood films. The centre figure represents a dividing form and the two right ones various states in plasmolysis. ($\times$ ca. 1,300).

Fig. 19. *Spirochæta (Leptospira) icterohæmorrhagiæ*, showing its appearance in life, as seen by dark-ground illumination. ($\times$ ca. 1,000).

Figs. 20 to 25. *Spirochæta anserina*. Figs. 20 to 22, stages in the elongation of short granular form into spirochætes, drawn from stained smears of the Malpighian tubule of *Argas persicus*, the intermediate host. ($\times$ ca. 1,000). Figs. 23 to 25, adult forms of *S. anserina*, a dried blood film of an infected fowl, stained with Giemsa. ($\times$ ca. 2,000).

(Figure 1, after Doflein; Figs. 2 and 19, after Wenyon; Figs. 3 and 4, after Gross, from Wenyon; Figs. 5 to 13 and 15 to 17, after Dobell; Fig. 14, after Hartmann and Schilling; Fig. 18, after Schellack; and Figs. 20 to 25, after Balfour.)

DESCRIPTION OF PLATE B (Figs. 26 to 31).

Figs. 26 and 27. *Spirochæta biflexa*. Drawing from a dark-field preparation of the adhesion phenomenon as applied to this species, showing (Fig. 26) a positive and (Fig. 27) a negative reaction ($\times$ ca. 1,000). After Brown and Davis (*Brit. J. Exp. Path.*, 1927, **8**, 398).

Fig. 28. Section through the intestine of *Ornithodoros moubata*, two hours after the tick had ingested blood containing *Spirochæta duttoni*, showing the penetration of the spirochætes into the epithelial cells, and their enrolment preparatory to segmentation into short forms. Stained Levaditi ($\times$ ca. 1,000). After Hatt (*Arch. Inst. Pasteur de Tunis*, 1929, **18**, 258).

Fig. 29. *Spirochæta gadi*. Section of kidney of *Gadus luscus*, showing spirochætes, an accidental blood parasite of the cod, in the renal tubules. Stained Levaditi ($\times$ 1,000). After Duboscq and Lebailly (*Arch. zool. exp. gén.*, 1913, **52**, 18).

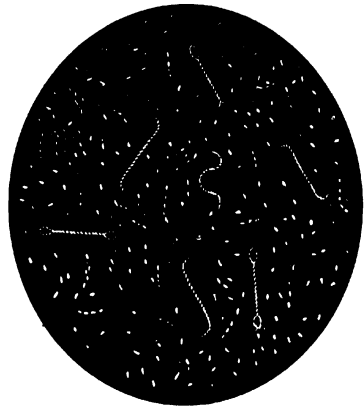
Fig. 30. Vincent's angina. Spirochætes and fusiform bacilli from a smear made from a lesion, showing various types that may be present. Stained Giemsa ($\times$ ca. 1,350). After Mühlens (Kolle and Wassermann, *Handb. d. path. Mikroorg.*, 2nd ed., **7**, 950).

Fig. 31. Ulcus tropicum. Transverse section through part of an ulcer, showing spirochætes and fusiform bacilli between the epithelial cells. Stained Levaditi ($\times$ ca. 1,000). After Keysselitz and Mayer (Kolle and Wassermann, *Handb. d. path. Mikroorg.*, 2nd ed., **7**, 950).

PLATE B.



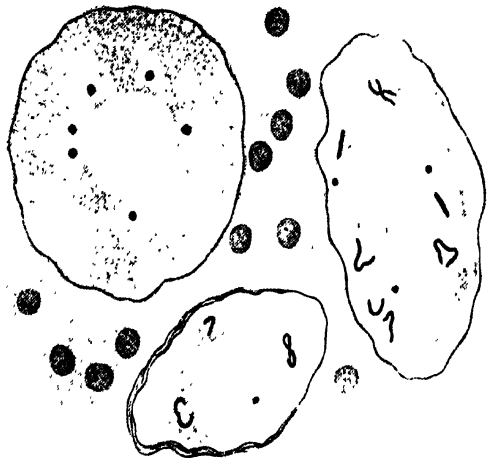
26



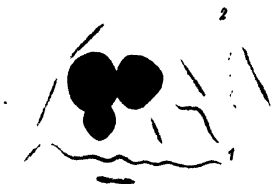
27



28



29



30



31

CHAPTER V. COMMENSAL SPIROCHÆTES.

BY EDWARD HINDLE,

THE BEIT RESEARCH FELLOW IN TROPICAL MEDICINE (WELLCOME
BUREAU OF SCIENTIFIC RESEARCH, LONDON).

THE commensal spirochætes, sometimes referred to as the mucocutaneous group, include a great variety of organisms commonly found living on mucous membranes or in the alimentary tract of man and animals, and also found associated with various ulcerative conditions of the skin. It is extremely difficult to decide the pathogenic significance, if any, of these various spirochætes, for in most instances, beyond their mere presence in the lesions, there is no very satisfactory evidence to show that they are anything more than mere saprophytes which find particularly suitable conditions for multiplication in dead and degenerating tissues. Their nomenclature is also extremely difficult, for at present there is no very certain method of distinguishing the various forms except by their dimensions and the nature of the spiral turns. The length is known to be variable, and there is evidence suggesting that the spirals may also vary, especially under artificial conditions, such as in cultures.

The names applied to the spirochætes mentioned in the following brief summary have been taken mainly from Noguchi's (1928) account of the group, and no attempt has been made to sort out the vexed question of their synonymy. It is only possible to refer to a few of the better known of these organisms; they are too numerous for all to be described individually in the present publication, as they have been found in the alimentary tract of practically every species of animal in which a search has been made for them.

Spirochætes of the Human Mouth (Figs. 7 to 13, facing p. 139).

A number of spirochætes have been described from the human mouth, but it is difficult to decide whether these represent different forms of the same spirochæte or distinct species. Dobell (1912)* made a careful study of this question and came to the opinion that there were at least three species: a large type, *S. buccalis*, 7 to 20 μ in length by 0.3 μ (Figs. 10 to 13); an intermediate form named by him *S. intermedia*, 6 to 14 μ in length by 0.25 μ ; and a small form, *S. dentium*, 4 to 18 μ in length by 0.2 μ , (Figs. 7 to 9). Noguchi (1928) considered there were seven or eight distinct species: two large types, *S. buccalis* and *S. vincenti*; one of intermediate

* Dobell used the generic name *Treponema*, but the species described by him are here referred to the genus *Spirochæta* in conformity with the system adopted in this volume.

size, called by him *S. macrodentium*, but probably identical with *S. intermedia*; and four small species, *S. mucosa*, *S. microdentium*, *S. dentium* or *orthodonta* and *S. skoliiodonta*. In addition, Hoffmann (1921) described a spirochæte, *S. trimerodonta*, of the leptospira type.

These spirochætes are generally abundant in the cavities of hollow teeth, or in the tartar on the surface, but even in clean and healthy mouths seem to be never entirely absent. At least three of them have been obtained in pure culture and may be assumed to be distinct species. These are as follows: *S. macrodentium*, probably synonymous with *S. intermedia*, was first cultured by Repaci (1911) and subsequently by Noguchi (1913), using the same method as that employed for *S. pallida*.

S. microdentium and *S. mucosa*, also cultured by Noguchi (1913), probably include *S. dentium*. They are indistinguishable morphologically and both produce odours in culture, but *S. mucosa* is distinguished by the fact that it produces mucin, and is able to survive when injected into the testis of a rabbit. It also exerts a pyogenous action when inoculated together with agar.

Two other forms have also been very much discussed, namely *S. vincenti* and *S. buccalis*.

✓ *S. vincenti* and *Vincent's angina* (Fig. 30, facing p. 140).

Vincent's angina is well known as an inflammatory condition of the tonsils and pharynx associated with the presence of large numbers of spirochætes (*S. vincenti* Blanchard, 1906) and fusiform bacilli in the affected regions. Similar associations of these two forms have been seen in hospital gangrene.

S. vincenti is usually present in small numbers in the normal mouth, and whether it bears any ætiological relationship to the conditions in which it is found in larger numbers is very difficult to decide. This organism has been cultivated by Tunncliffe (1905) on serum-agar, who considers that the fusiform bacilli represent a stage in the development of the spirochætes. The possibility of its relationship to the fusiform bacilli, with which it is commonly associated, is discussed in Chapter XI.

Bronchial spirochætosis.

This is another condition supposed to be due to the presence of spirochætes which are considered by some authors to be identical with *S. buccalis*, by others with *S. vincenti*, and by a third group of writers as a distinct species, *S. bronchialis*. Castellani (1906, 1909) was the first to describe this as a distinct affection, and subsequently a very extensive literature on the subject has not rendered the question any clearer as to the causal relationship between the spirochætes and various putrid types of bronchitis and empyema, lung gangrene and lung abscess. These conditions are associated with the presence of fusiform bacilli and cocci in addition to spirochætes, and experiments by D. T. Smith (1928) suggest that at least four organisms are necessary to produce experimentally a

certain type of lung abscess in rabbits. Smith also found that the isolation of spirochætes from sputum was simplified by inoculating the material into the groin of a guinea-pig. After two or three passages the gas-forming bacteria could generally be eliminated or sufficiently reduced in number to enable the spirochætes to be isolated in the usual manner.

This observer, as the result of an extensive study of the subject, came to the conclusion that pulmonary gangrene, most cases of pulmonary abscess, certain types of unresolved pneumonia, and bloody bronchitis, putrid bronchitis and chronic bronchiectasis, were not separate disease entities, but different manifestations of infection with a group of anaerobic organisms acting in symbiosis. This group is said to include: (1) *S. macrodentium*, *S. microdentium*, *S. vincenti* and *S. bronchialis*; (2) fusiform bacilli; (3) vibrios; (4) cocci. Similar organisms are normally present in the gums of patients suffering from pyorrhœa, and pulmonary abscesses may be obtained by the intratracheal inoculation of this material into experimental animals (Smith, 1927).

It should be noted that there is no general agreement among authors as to the species of spirochætes occurring in this condition, and although the frequent, though by no means constant, success of treatment with organic arsenical compounds is considered by some to support the hypothesis that these organisms are of pathogenic significance, there are so many disagreements on almost every question involved, that many authorities have considerable hesitation in accepting the existence of bronchial spirochætosis as a distinct infection.

Those interested in the subject will find the arguments in support of the pathogenic significance of these organisms discussed at length by Delamare (1925).

Intestinal Spirochaetes.

These are present in the intestines of men and animals, and in some cases seem to be identical with mouth spirochætes (Mühlens, 1907). The two most common species are *S. eurygyrata* and *S. stenostrepta*, both of which occur in normal human fæces (Werner, 1909). The former is a short spirochæte with only two flat spirals often forming an S-shape; whilst the latter has two to six close spirals. A longer form resembling *S. recurrentis* has been described by Macfie and Carter (1917) and named *S. intestinalis*. In addition, various other species have been recorded. Hogue (1923) obtained specimens of *S. eurygyrata* varying in length from 4 to 56 μ . These spirochætes were grown in a medium composed of 0.3 c.cm. dilute pig-serum (serum 1, water 3), in 15 c.cm. of 0.85 per cent. saline solution. Small drops of a suspension of the spirochætes in this medium were examined until one was found containing only one organism. This was then removed by means of a micropipette, inoculated into a culture tube and grown at 35° C.

In addition to variations in length, considerable differences were also observed in the number and arrangement of the spirals. Parr (1923) came to the conclusion that until better evidence was forthcoming it was

advisable to consider all intestinal spirochætes as belonging to a single species. Delamare and Achitouv (1923) came to a similar conclusion as a result of their observations on intestinal spirochætes cultivated in serum bouillon. Le Dantec (1903) observed that in the fæces of a dysenteric patient a kind of membrane was formed by a plentiful growth of these spirochætes, and advanced the hypothesis that they were of pathogenic significance. Broughton-Alcock (1923), among others, has also expressed the opinion that *S. eurygyrata* has some significance in certain types of dysentery, whilst *S. stenostrepta* is non-pathogenic. The work of Sanarelli on the intestinal spirochætes of guinea-pigs has been referred to elsewhere (see p. 117).

Ulcus tropicum and Spirochætes found in the Skin (Figs. 14 and 31).

The association of various saprophytic spirochætes with *S. pallida* and *S. pertenuis* has been described in Chapter VII. In addition, various chronic ulcerations of the skin often contain spirochætes generally associated with fusiform bacilli and other bacteria. *Ulcus tropicum* is one of the best known of these conditions and is characterized by a chronic ulceration of the skin which affects persons in tropical countries. Vincent (1896) was the first to notice the presence of a spirochæte in this condition and considered it the same as that occurring in Vincent's angina.

Prowazek (1907) named the spirochæte found in these ulcers *S. schaudinnii*, and Keysselitz and Mayer (1909) considered that the ulceration was caused by this organism. The spirochæte is described as being 6 to 35 μ in length, the average being 12 to 25 μ , with spirals resembling those of *S. recurrentis*. Sections of ulcers show the presence of spirochætes, fusiform bacilli and other bacteria on the surface; below this there are only spirochætes and fusiform bacilli, whilst in the deepest layers spirochætes alone are present. E. C. Smith (1930) has cultured the organisms from tropical ulcers in West Africa and reproduced the disease experimentally by the inoculation of these mixed cultures after growth for several months in Noguchi-Wenyon medium at 37° C. The cultures were still virulent after repeated passages, but as they contained at least four types of organisms, spirochætes, spirilla, fusiform bacilli, and bacteria, it is impossible to say whether any particular one of them was pathogenic. It is possible that all four are necessary to produce the pathological conditions, but this can only be decided by the isolation in pure culture of these organisms and making inoculation experiments with each alone, and in various combinations.

The question of the identity of *S. vincenti* from the mouth with this organism also can only be decided by comparison of pure cultures.

S. aboriginalis, described by Cleland (1909) from a case of granuloma inguinale in Australia; *S. minima*, described by Aragão and Vianna (1913) from a chronic ulcer; *S. balanitidis* (Fig. 14), found by Hoffmann and Prowazek (1906) in a case of balanitis erosiva circinata, and many other forms possibly identical with *S. vincenti* have been recorded. *S. noguchii*

(Strong, 1925) was described from a type of skin ulcer in South America, consisting of nodular, warty growths about 1 to 3 cm. in diameter. Spirochætes 14 to 20 μ in length were abundant in the lesion and were found to stain more deeply than *S. vincenti*, from which this form is considered distinct.

S. phagedenis, cultured by Noguchi (1912) from phagedenic ulcers, is another spirochæte with very doubtful affinities. In addition, spirochætes have been recorded in various cancers, such as *S. microgyrata* described by Loewenthal (1906). They are occasionally found invading mouse carcinomata (Borrel, 1905), and when these are transplanted to other mice the spirochætes develop in the new growths.

Spirochætes found in the Genitalia.

A number of these organisms have been described from the genitalia of normal individuals, but, as in the case of other saprophytic spirochætes occurring in man and animals, there is considerable uncertainty as to their nature. Three types are commonly present and correspond with those found in the mouth.

S. refringens is a large, thick spirochæte first noticed by Schaudinn and Hoffmann (1905). It may readily be distinguished from *S. pallida* by its greater thickness and flexibility. *S. calligyra*, an intermediate form, is often present in smegma, but was isolated by Noguchi (1913) from a condyloma. It requires anaerobic conditions for growth in culture. This spirochæte is 9 to 12 μ in length and 0.35 to 0.40 μ in thickness, with five to eight rather deep spirals. *S. genitalis*, the smallest form, according to Noguchi (1928) resembles *S. microdentium* of the mouth, but may be distinguished by its immunological reactions and also by the fact that, unlike the latter, it does not produce odour in cultures.

Castellani (1915), under the name *S. urethrale*, described a very fine spirochæte 6 to 12 μ in length, with many regular spirals, as occurring in a purulent urethral discharge.

Macfie (1916, 1917) also described a spirochæte of the relapsing-fever type from the vaginal discharge of a case at the Accra Hospital, and a larger form from the male urethra. These were named respectively *S. vaginalis* and *S. urethræ* and were supposed to be of pathogenic significance. *S. pseudopallida* Mulzer (1905) and *S. gangrænosa nosocomialis* Rona (1907) have been described from ulcers in the genital region.

REFERENCES.

- ARAGÃO, H. DE B. & VIANNA, G., 1913, *Mem. Inst. Oswaldo Cruz*, **5**, 211.
 BORREL, A., 1905, *C.R. Soc. Biol.*, Paris, **58**, 770.
 BROUGHTON-ALCOCK, A., 1923, *Proc. R. Soc. Med.*, **14** (Sect. Trop.), 46.
 CASTELLANI, A., 1906, *Lancet*, Lond., i, 1384; 1909, *Brit. Med. J.*, ii, 782; 1915, in Castellani and Chalmers's *Manual of tropical medicine*, 3rd Edit., Baillière, Tindall & Cox, London.
 CLELAND, J. B., 1909, *J. Trop. Med.*, **12**, 143.
 DELAMARE, G., 1925, *Spirochètoses respiratoires stomatogènes*, Masson et Cie, Paris.
 DELAMARE, G. & ACHITOUV, 1923, *C.R. Acad. Sci.*, Paris, **177**, 1331.

- DOBELL, C. C., 1912, *Arch. Protistenk.*, **26**, 117.
 HOFFMANN, E., 1921, *Zbl. Bakt.*, Abt. I, Orig., **86**, 134.
 HOFFMANN, E. & PROWAZEK, S. VON, 1906, *Zbl. Bakt.*, **41**, 741, 817.
 HOGUE, M. J., 1923, *J. Exp. Med.*, **36**, 617.
 KEYSSSELITZ, G. & MAYER, M., 1909, *Arch. Schiffs- u. Tropenhyg.*, **13**, 137.
 LE DANTEC, 1903, *C.R. Soc. Biol.*, Paris, **55**, 617.
 LOEWENTHAL, W., 1906, *Berl. klin. Wschr.*, **43**, 283.
 MACFIE, J. W. S., 1916, *Ann. Trop. Med. Parasit.*, **10**, 305 ; 1917, *Parasitology*, **9**, 274.
 MACFIE, J. W. S. & CARTER, H. F., 1917, *Ann. Trop. Med. Parasit.*, **11**, 75.
 MÜHLENS, P., 1907, *Z. Hyg. InfektKr.*, **57**, 405.
 MULZER, P., 1905, *Berl. klin. Wschr.*, **42**, 1144.
 NOGUCHI, H., 1912, *J. Exp. Med.*, **16**, 261 ; 1913, *ibid.*, **17**, 89 ; 1918, *ibid.*, **27**, 667 ;
 1928, Article, *The Spirochætes*, in Jordan and Falk's *The newer knowledge of bacteriology and immunology*, Chicago University Press.
 NOGUCHI, H. & KALISKI, D. J., 1918, *J. Exp. Med.*, **28**, 559.
 PARR, L. W., 1923, *J. Infect. Dis.*, **33**, 369.
 PROWAZEK, S. VON, 1907, *Arb. GesundhAmt.*, Berl., **26**, 23.
 REPACI, G., 1911, *C.R. Soc. Biol.*, Paris, **70**, 784.
 RONA, S., 1907, *Verh. deuts. dermat. Ges.*, **9**, 471.
 SCHAUDINN, F. & HOFFMANN, E., 1905, *Deuts. med. Wschr.*, **31**, 1728.
 SMITH, D. T., 1927, *Arch. of Surg.*, Chicago, **14**, 231 ; 1928, *Tubercle*, Lond., **9**, 420.
 SMITH, E. C., 1930, *Proc. R. Soc. Med.*, **24**, 217.
 STRONG, R. P., 1925, *United Fruit Co. Medical Dept. 14th Annual Report*.
 VINCENT, H., 1896, *Ann. Inst. Pasteur*, **10**, 488.
 WERNER, H., 1909, *Zbl. Bakt.*, Abt. I, Orig., **52**, 241.

CHAPTER VI. BLOOD SPIROCHÆTES.

BY EDWARD HINDLE,
THE BEIT RESEARCH FELLOW IN TROPICAL MEDICINE (WELLCOME
BUREAU OF SCIENTIFIC RESEARCH, LONDON).

Introduction.

Definition. Spirochætes normally inhabiting the blood of man or animals, in which they often produce a relapsing type of fever. They are generally transmitted from one host to another by the agency of blood-sucking arthropods, such as ticks or lice.

The blood spirochætes of man. Spirochætes have been found in the blood of man in most parts of the world, with the exception of Australia, and are responsible for the production of various types of relapsing fever. The type of fever, the nature of the symptoms, the method of transmission and the serological characters, present numerous variations, and the causative organism from each locality has often been given a specific designation. It should be emphasized that all these relapsing-fever spirochætes are merely local varieties of one widely distributed species, *Spirochæta recurrentis*, that has become adapted to different methods of transmission in various localities. The different races, however, are generally known by their various designations (e.g. *S. duttoni*, *S. persica*, *S. berbera*, &c.), and for convenience these names have been retained, but strictly speaking they are all merely physiological varieties of *S. recurrentis*, and cannot be distinguished on any morphological basis (see Macfie, 1916 ; Macfie and Yorke, 1917 ; Wenyon, 1926 ; Mühlens, 1930, &c.).

General Account.

The spirochætes of relapsing fever are present in the blood during the febrile attacks, and can generally be seen without much difficulty, especially during the first attack. As a rule, the number of parasites in the blood becomes smaller in succeeding relapses, and it is often necessary to use thick films in order to detect them. During the intervals between the febrile attacks, spirochætes have very rarely been found, and either the spiral forms are extremely scanty, or the spirochætes are present in an unrecognizable form, for the blood has been shown to be infective during these seemingly negative periods.

The number of febrile attacks varies considerably with different races of spirochætes, and even with the same race in different individuals. As a general rule, relapsing fevers transmitted by ticks seem to be more severe and show more relapses than those in which lice are vectors. In the case of *S. duttoni*, transmitted by *Ornithodoros moubata*, the number of febrile attacks is generally five or six, but as many as eleven have been recorded. The typical *S. recurrentis* of Europe, rarely shows more than two or three febrile attacks.

The duration of the febrile period is generally two or three days and the apyretic intervals vary from one or two days up to as long as three weeks. Each febrile attack terminates in a crisis, when the spirochætes agglutinate and very rapidly fragment into small particles. At this stage very large numbers are ingested by the leucocytes and in a few hours the circulating blood, which may have contained almost as many parasites as red cells, will become negative. The disappearance of the spirochætes is caused by the development of antibodies, whose presence can be demonstrated, but as mentioned previously these antibodies are extremely specific, and are only able to give complete protection against the same race of spirochætes, at the same stage of development, and with an infection produced in the same manner.

Thus the antibodies produced after the first febrile attack will not protect the body against spirochætes of the second attack. Also a patient recovered from relapsing fever produced by the bites of ticks, can be reinfected by the inoculation of the same race of spirochætes after passage through a mouse. Under these circumstances the difficulty of deciding how to subdivide these organisms into distinct races will be readily understood.

MORPHOLOGY (Figs. 18 and 20 to 25, facing p. 139).

The spirochætes of all relapsing fevers when present in the blood show great variation in size, but the average length is about 15μ , and diameter between 0.2 and 0.3μ . The majority of forms fall between 10 and 20μ in length, but chains of individuals are sometimes observed which may be as long as 80 to 100μ . The extremities are slightly pointed. The length of each spiral turn is between 2 and 3μ , so that an organism 15μ long will show five to seven turns. Although these are the common blood forms it is possible to find very short individuals with only one or two spiral turns, indicating either unequal or extremely rapid transverse division, or development from coccoid bodies.

Dividing forms can easily be found at the beginning of a febrile attack and can be recognized, apart from their length, by a constriction about the middle of the organism. Division is invariably transverse and the two daughter-spirochætes often remain connected for some time by their drawn-out extremities.

Involution forms of various kinds may often be seen, especially when the spirochætes are disappearing from the blood. Some of these appearances have been interpreted as developmental forms, but no satisfactory evidence has been brought forward to support this view. The most common involution form is the appearance of one or more swellings, sometimes terminal, but more often along the length of the spirochæte (Fig. 18). The swelling of the periplast may be the result of cytolysis, and the appearance of one or two dark-staining particles seems to be the result of the remaining contents of the spirochæte being compressed into the swelling. These large cysts have sometimes been regarded as spores, but

they would seem to be the result of cytolysis. Another type of so-called spore formation has been described by several observers, and in particular by Dutton and Todd (1907), Leishman (1920), Balfour (1911¹), and Hindle (1912²). The whole spirochæte segments into a number of small granules, about 0.2μ in diameter, which become free in the blood-stream. The same process has been observed in cultures of spirochætes (Noguchi, 1912) and in the intermediate host (see below, p. 159). These small granules, known as spores, coccoid bodies, or Leishman's granules, are supposed by some authors to represent a phase of the spirochæte, and by others to be merely a product of degeneration (Figs. 20 to 22 and 28).

The living organisms are actively motile and by means of spiral movements of their body are able to penetrate tissue-cells. They are present in the cerebrospinal fluid as well as in the blood, and also invade various tissues, including the brain. Their power of penetration is such that they are able to traverse uninjured mucous membranes.

Special organs of locomotion, such as lateral flagella or cilia, have been described, but all such appearances are probably the result of artefacts. Dividing forms frequently retain a drawn-out extremity for some considerable period after division, and this may be regarded as a terminal flagellum, though ultimately it disappears. The short granular forms are also stated to have a flagellum (Seguin, 1930).

STAINING.

Spirochætes are notoriously difficult to stain, but the blood forms may be coloured more readily than the finer tissue spirochætes and leptospiras. The most useful stain for ordinary purposes is Romanowsky or one of its many modifications, and may be used for either thick drops or thin smears.

A thick film is prepared by spreading two or three drops of blood in the middle of a slide so that they occupy an area of about three-quarters of an inch diameter. The film is then allowed to dry, *without heating*, and when dry is passed through a flame once or twice and then de-hæmoglobinized by washing in distilled water. When all the hæmoglobin has been dissolved out, the film is ready for staining. Thin smears are prepared in the ordinary way, but before staining should be fixed for 5 to 10 minutes in absolute alcohol, and the alcohol washed off with distilled water. It is essential for good results that the alcohol used for fixing should be washed off and not merely allowed to evaporate. The latter and more usual method often leaves a fine deposit which takes the stain and obscures the preparation.

In my experience, ordinary Romanowsky stain, as recommended by Stephens and Christophers (1908), gives quite as good results as Leishman, Giemsa, or any other of the numerous modifications of this staining method. The stain is merely a mixture of equal parts of 1 in 20,000 aqueous eosin (BA or similar brand), and 1 in 2,000 polychrome methylene blue. The solutions are kept separate and mixed immediately before use. The films are allowed to stain for one or two hours, or even left over-night

if kept inverted in a covered dish to prevent evaporation, and then rinsed in distilled water and allowed to dry. With this method spirochætes stain a dark purplish red and are readily distinguished from artefacts by their well-defined form and tapering ends. As in the case of all spirochætes it is essential to see well-defined extremities in order to distinguish them from the extremely realistic artefacts frequently seen in stained films.

Very many other staining methods have been used for these organisms, full particulars of which may be found in the monographs by Collier (1926) or Pettit (1928), but reference should be made to the various silver impregnation methods which are extensively used in the demonstration of these organisms.

Fontana's Method.

Fontana's method, or one of its numerous modifications, is most commonly used for the demonstration of spirochætes in dried films and is applied as follows (Fontana, 1912). The film is made as thin as possible and then washed for one to two minutes in a solution composed of: glacial acetic acid, 1 part; formol, 2 parts; distilled water, 100 parts. The film is then washed in running water for half a minute and afterwards mordanted in the following solution: phenol, 1 part; tannic acid, 5 parts; distilled water, 100 parts. This solution is poured on to the film and the slide then warmed over a flame until it steams. It is kept in this mordant for about half a minute, then rinsed in distilled water and ammoniacal silver nitrate poured on to the film. The slide is again warmed for about half a minute, after which the film is washed, dried and mounted in Canada balsam. The ammoniacal silver nitrate is prepared by adding liquid ammonia to a 0·25 per cent. solution of silver nitrate in distilled water until the precipitate which first forms is redissolved. Finally, silver nitrate solution is added drop by drop, until the liquid becomes opalescent.

This method results in the spirochætes being coated with a deposit of metallic silver and as a result they stand out very distinctly as dark filaments, which are much thicker than the living organisms.

Dobell's Modification of Levaditi's Silver Impregnation Method.
(Wenyon, 1926.)

This method is applicable to the staining of spirochætes in tissues, which is very difficult to accomplish with any of the ordinary staining methods. The tissues are fixed in 4 per cent. formaldehyde solution for at least 1 to 3 days, but may be kept in this solution indefinitely before being stained. They are then transferred to 95 per cent. alcohol for 24 hours, and afterwards through graded alcohols to distilled water until all traces of alcohol are removed. The tissues are then soaked in a 0·25 to 0·5 per cent. solution of silver nitrate in distilled water for 18 to 24 hours in the dark at 37° C. They are afterwards washed in changes of distilled water for an hour until the wash water is free from silver nitrate, and then soaked in a 0·5 to 1 per cent. solution of hydroquinone in 50 per cent. alcohol for 18 to 24 hours at room temperature. Finally, the tissues

are washed in 70 per cent. alcohol, dehydrated and embedded in paraffin in the usual manner. The sections, after having been fixed on slides, are washed with xylol and may then be mounted in Canada balsam or may be counter-stained by any of the ordinary staining methods. The spirochætes stand out very distinctly as dark filaments, owing to the deposition of silver on their surface, and as a rule can readily be distinguished from any other structures that may take the stain in a similar manner.

CULTIVATION.

Noguchi (1912) was the first author to succeed in maintaining a strain of blood spirochætes by continued subculture. He employed a similar method to that used for the growth of *S. pallida*, and cultivated four strains of spirochætes. A piece of fresh tissue, usually rabbit-kidney, is placed in a sterile test-tube and a few drops of citrated blood containing spirochætes is then added. About 15 c.cm. of sterile ascitic or hydrocele fluid is quickly poured into the tube, followed by a layer of sterile paraffin oil in some tubes, while others are left uncovered. The tubes are incubated at 37° C. Subcultures are effected by using 0.5 c.cm. of the original culture instead of infected blood, but it is advisable to add a little fresh blood at the same time. Some strains were found to attain their maximum growth on the seventh day and others on the tenth, but subcultures could be made from the fourth day onwards.

Various modifications have been introduced and it is now possible to culture these organisms in a variety of ways, but whichever method is adopted unexpected failures will be met with. The general conditions necessary for their growth are as follows :

(1) A limited supply of oxygen obtained in the presence of coagulated albumin, such as egg-white, serum, tissues, &c., in long narrow tubes, by covering the surface of the medium with paraffin oil or vaseline, which also prevents evaporation.

(2) A medium containing albumin obtained by the addition of fresh serum from a horse or rabbit, diluted with saline.

(3) The presence of blood, or pieces of fresh tissue, or embryonic tissue.

(4) The pH value is of vital importance and must be adjusted to the optimum for the organism being cultivated.

Lapidari and Sparrow (1928), who have studied this question, found that a modification of Ungermann and Galloway's method described by Bruynoghe (1928) gave the best results. A small quantity, 1 c.cm., of egg-yolk is placed in a narrow sterile tube and coagulated into a cup-shape by keeping the tube slanting whilst rotating it in boiling water. Rabbit-serum diluted to one-sixth with either glucose Ringer solution*, or preferably with Hartley's broth, is then added, and the medium immediately covered with a layer of sterile paraffin about 1 cm. deep and the tube then heated at 56 to 58° C. for one hour. Before using the tube, a drop of sterile blood (man, rabbit, mouse, &c.) is added, and the tube rotated

* Glucose 1 gm., NaCl 9 gm., CaCl₂ 0.24 gm., NaHCO₃ 0.1 gm., water 1,000 c.cm.

to distribute the corpuscles evenly through the medium. A fibrinous reticulum is formed which afterwards contracts. The addition of blood is unnecessary when it is intended to isolate spirochætes from blood, as enough is introduced when seeding the culture-tube. After being seeded the tubes are kept at 36 to 37° C., and two or three days' incubation is sufficient to show several spirochætes in each microscopic field. The cultures grow until the eighth day, and are especially rich at the bottom of the tube. Occasionally these cultures show relapses, the spirochætes being abundant on the fifth and seventh days, then disappearing until the sixteenth day and later attaining their maxima from the twentieth to the thirty-third day. It is best to make subcultures every seven days. The addition of either glucose or lævulose to the medium seems to be necessary and produces increased motility of the organisms. The paraffin helps to keep the pH constant, and this should be in the neighbourhood of 7·4.

The form of the spirochæte in cultures is the same as when present in the blood circulation.

PATHOGENIC ACTION.

The different strains of blood spirochætes show considerable variation in their pathogenic action in experimental animals. Generally speaking, however, monkeys and rodents are particularly susceptible and can be infected by the percutaneous, subcutaneous or intraperitoneal inoculation of blood from an infected patient. Although as a routine method the subcutaneous method is generally employed, contrary to what one might have expected, animals infected by percutaneous methods seem to show the most severe infections. Tomioka (1924) records that mice infected with European relapsing fever showed a mortality rate of 70 per cent. when this method was used, whilst those inoculated subcutaneously or intraperitoneally had a mortality rate of 42 per cent. The method employed is to leave a drop of infected blood on the shaved skin for about half an hour and to wash it off after drying.

Although all strains of relapsing fever that have been tested, with the exception of Brazilian strains, are pathogenic to monkeys, the different races show considerable variations in their effects in other animals. *S. recurrentis*, in its typical European form, can be inoculated direct from a patient into a monkey and produces a typical infection which can be transmitted from the monkey to rats or mice. It is very difficult, as a general rule, to infect these rodents directly from a human case, but individual cases vary in this respect, and both rats, mice and guinea-pigs have been successfully infected by the inoculation of blood from patients. This strain of relapsing fever generally produces only one or two febrile attacks in mice or rats, and its maintenance in a laboratory requires very constant attention.

The Indian (*S. carteri*) and North American (*S. novyi*) strains, although resembling the typical *recurrentis* in their pathogenicity, are inoculable direct into rats and mice.

The Chinese strain, when inoculated intraperitoneally into the grey squirrel (*Sciurus vulgaris*), produces a slight attack, but ordinarily there are no relapses. If these animals are splenectomized, however, the initial attack is much more severe and is followed by one or two relapses (Meleney, 1927). In Shantung I inoculated hamsters, *Cricetulus griseus*, and also mice with infected blood from a Chinese case of relapsing fever, and the animals showed spirochætes for two or three days, but there were no relapses and the strain could not be maintained in these animals.

S. berbera, the spirochæte of North African relapsing fever, can be inoculated directly from man into monkeys, but the resulting infection cannot be carried on in these animals; also it is very difficult to infect rats and mice, and Sergeant and Foley found it necessary to use newly born mice in order to obtain successful *passage*. A Tunisian strain, however, was inoculated directly into rabbits and various passages were effected in these animals. The West African strain on the Gold Coast has been successfully inoculated into monkeys, and into black and white rats, in which it shows no relapses; pouched rats were much more susceptible to the infection than any other animals studied, and showed relapses. Guinea-pigs and rabbits were refractory (Selwyn-Clarke, Le Fanu and Ingram, 1923).

The relapsing fever at Tonkin has been studied by Mathis and Leger (1911¹), who found that monkeys (*Macacus* sp.) and white mice were susceptible to the infection but rarely showed any relapses. Wild rats (*Mus decumanus*), rabbits and guinea-pigs were resistant.

All the above-mentioned strains of relapsing fever are generally transmitted by the agency of lice and show some agreement in being only mildly pathogenic to laboratory animals. The following strains are transmitted by ticks and most of them can be maintained in laboratory animals with much greater ease.

S. duttoni, probably the best-known strain in laboratories, is the name given to the spirochæte of African tick fever, and in nature is transmitted by the human tick *Ornithodoros moubata*. This strain of spirochætes will readily infect a wide range of animals, including the following: monkey, horse, goat, sheep, rabbit, dog, guinea-pig, squirrel, bat, European hamster, rat and mouse. The horse, goat, sheep and rabbit have cryptic infections, but their blood can be shown to contain spirochætes by subinoculation into rats. Monkeys and white rats or mice are the most convenient animals to employ and they can easily be infected by the inoculation of blood from a relapsing-fever patient or from an infected animal, also by the inoculation of the infective contents of the ticks which carry the disease. Infection can also be produced by their bites, but for reasons which will be obvious when the details of transmission are considered, this is not such a certain method as inoculation of their contents.

S. crociduræ, the name given to the spirochæte of the Senegal strain of relapsing fever, is now considered to be the same as *S. duttoni* (Mathis,

1928). This strain occurs naturally in a fairly high percentage of animals, for out of 30 *Crocidura stamplii*, 6 were infected ; 3 *Mus decumanus* out of 30 ; 4 *Mus coucha* out of 20 ; and 4 *Golunda campanæ* out of 30 (Leger, 1923). Also this spirochæte will readily develop in monkeys, white rats and mice, and other common laboratory animals.

S. hispanica, the Spanish relapsing fever spirochæte, has been successfully transmitted to a variety of animals, in some cases by inoculation of infected blood, and in others by the contents of infected ticks, *Ornithodoros maroccanus*. Monkeys, young dogs and cats, rabbits, guinea-pigs, and especially rodents, have been infected, but although the insect host, *O. maroccanus*, lives in association with pigs, hitherto all attempts to infect this animal have failed. In the gerbil, *Meriones shawi*, this strain gives rise to an infection without visible spirochætes, yet the merest trace of its blood will infect a guinea-pig, even by way of the conjunctiva.

S. venezuelensis and *S. neotropicalis*, occurring in Central and South America, can be inoculated directly from man into rats and mice. Unlike most tick-borne spirochætes, these strains seem to be unable to persist in American species of monkeys, and dogs, rabbits and guinea-pigs are also refractory. A Brazilian strain of relapsing fever, studied by Brumpt, Bourrol and Guimarães (see Brumpt, 1927, p. 62), was inoculated into monkeys, rats and mice with negative results, but this seems to be the only record of a human blood spirochæte unable to persist in another vertebrate.

Pathological effects. The course of the infection in experimental animals is very similar in the case of all the above-mentioned strains. An incubation period of 1 to 10 days, depending mainly on the dose, method of inoculation and virulence of the strain, is followed by the appearance of spirochætes in the blood, accompanied by a sudden rise in temperature. Generally, they remain in the blood for two or three days, after which the febrile attack ends by lysis, and the spirochætes disappear very rapidly, and the body temperature falls to normal or subnormal.

In the case of animals infected with European, Chinese and North American strains, this first attack may be the only one developed, but commonly after an interval of a few days there is a second febrile attack, or *first relapse*, resembling the initial one in its history. A succession of relapses may be observed, gradually diminishing in severity in non-fatal cases, and eventually the host recovers from the infection. The disease is often fatal in animals and in such cases the parasites in the blood, instead of diminishing, may remain constantly present in large numbers until death.

The pathological changes in infected animals vary considerably with different strains and also in different individuals. The most constant features are anæmia and loss in weight. A marked leucocytosis always occurs when the parasites are about to disappear, sometimes rising to 60,000 per c.cm. At autopsy the heart-muscle is often degenerated. Exudate may be present in the pleural, pericardial and peritoneal cavities. The spleen is always much enlarged, deeply congested and very soft. In

acute cases, numerous hæmorrhagic infarcts occur throughout the organ, and necrotic areas of varying size are often present. The liver is often enlarged, with small hæmorrhages, and small necrotic regions occur in long-standing cases. The lungs become œdematous and show hæmorrhagic infarcts, and small sub-pleural petechiæ are often seen. The lymphatics are frequently hæmorrhagic. The bone-marrow becomes dark purple in colour and is softened to a semi-fluid consistence (Breinl and Kinghorn, 1906 ; see also Bykowa, 1926).

According to Levaditi and Manouélian (1907) the spirochæte multiplies only in the blood-system and not in the body-cells, but they are undoubtedly able to penetrate into all parts of the body, and can even pass through the placenta into the embryo (Breinl and Kinghorn, 1906 ; Nattan Larrier, 1911). It is also known that they invade the nervous system, where they persist long after all other parts of the body have become free from the infection (Buschke and Kroó, 1923).

The exact manner in which spirochætes act on their host is not obvious, and there is no very satisfactory evidence incriminating endotoxins, except Schilling and Rondoni's (1913) observations that suspensions of *S. duttoni* kept at 37° C. for 4 hours became toxic to mice, although doses of 0.25 c.cm. were not always fatal within 18 hours.

The multiplication of spirochætes in the blood results in the capillaries becoming obstructed by polymorphonuclear leucocytes and masses of spirochætes, especially in the liver, where the macrophages may be seen to contain many ingested parasites. According to Sanarelli (1927) the spirochætes act by merely reducing the natural resistance of the body, especially by their effect on the liver, and thus favour the development of secondary infections. This view has been discussed more fully elsewhere (p. 117).

IMMUNITY.

The immunity following an attack of relapsing fever is generally of short duration, and in human beings reinfection may occur after an interval of one to two years. The duration varies considerably, however, being longer after a severe attack and shorter after a mild one. The immunity is very specific ; for example, a patient who has recovered from an attack of European relapsing fever can be infected with any other variety of relapsing fever. This is the basis of the cross-immunity tests which have been so extensively used for the multiplication of the number of ' species ' of relapsing-fever spirochætes and the creation of a bewildering number of names. As indicated above (p. 148), this reaction will distinguish spirochætes of different relapses of the same strain, the same spirochæte after passage through different hosts, &c., and is far too delicate to be a reliable guide in the subdivision of relapsing-fever spirochætes.

The serum of a recovered man or animal contains specific agglutinins and lysins. By the use of a suitable antigen, such as an extract of the spleen of an infected mouse, fixation of complement may be obtained after the first relapse (Zlatogoroff, 1923).

The importance of the reticulo-endothelial system in the development of protective substances against spirochætal infections has been very clearly shown in the case of white mice infected with *S. recurrentis*. Whereas the mortality in normal mice infected with this spirochæte was only 3·8 per cent., in mice that had been splenectomized and the endothelial system blocked with colloidal iron, the mortality was more than 90 per cent. (Kritschewski and Rubinstein, 1927). Consequently, splenectomized animals are much more susceptible to spirochætal infections than normal individuals.

BRAIN INFECTIONS.

The persistence of relapsing-fever spirochætes in the brains of animals for some months after apparent recovery from infection was first noticed by Buschke and Kroó (1923), working with a European strain of relapsing fever in rats and mice. In the case of mice the invasion of the brain was not accompanied by any histological changes, but in rats the animals remained ill after spirochætes had disappeared from the blood. Some of these rats showed lesions of the posterior column which involved areas into which fibres from posterior nerve-roots entered. These observations on the persistence of spirochætes in the brain have been repeatedly confirmed. Tomioka (1924), employing a similar strain in mice, found that up to 21 days after inoculation the brains of all contained spirochætes, but after 36 days only one out of five. The effects of salvarsan treatment suggested that when a large dose was given at an early stage of infection, the number of brain infections was greatly reduced.

Although Buschke maintained that brain infections persist after salvarsan treatment, his results have been criticized by Prigge (1926), who found that, if treated with a sufficient dosage of salvarsan, especially in early stages, the infection in mice could be completely eradicated, and similar results were obtained by Schreus and Weisbecker (1926). Johannessohn (1926) showed that if the treatment is delayed and the spirochætes have had time to become distributed through the body, treatment will sterilize the blood but not the brain.

Different strains of spirochætes give varying percentages of residual brain infection (Kritschewski, 1927), and recently isolated strains seem to produce a much higher percentage than strains maintained in experimental animals for long periods. Prigge and Rothermundt (1928) found that by inoculation of the former type practically every mouse showed a residual brain infection, whilst with the latter only 3 out of 40 mice showed any persistence of spirochætes in the brain. In common with most other German authors, they explain the presence or absence of brain infections as a result of the comparatively feeble blood-supply in that region; accordingly, unless there is a high concentration of antibodies or chemotherapeutic agents in the blood, spirochætes are not destroyed.

Reinfection seems to increase the chance of the brain becoming infected (Heronimus, 1928). According to Rosenholz, Owsjannikowa and Treflow

(1928), by passing a needle into the brain of a mouse and then inoculating the animal in the ordinary way with either *S. recurrentis* or *duttoni*, infection of the nervous system was invariably obtained. Lebedjewa (1929), on the other hand, found that brain injury had no influence whatever on the percentage of brain infections.

Levaditi and Anderson (1928 and 1929), working with a strain of *S. duttoni* in mice, found that the infection persisted in the brain in an invisible form, for although infected nervous tissue was carefully examined, no trace of spirochætes could be discovered. Nevertheless, the virus was not filterable, from which these authors assume that it is intimately associated with the nerve-cells. Further experiments have shown that this stage is very easily adsorbed in filters with pores even up to 15 to 21 μ in diameter, but will pass through filters with pores 25 to 36 μ in diameter.

The brain infection is not resistant to immune-bodies present in the blood, and also will produce blood infection when animals are inoculated intracerebrally.

It is evident, therefore, that relapsing-fever spirochætes in some stage can persist in the brain long after they have disappeared from all other parts of the body, in the case of *S. duttoni* up to 191 days after inoculation. Moreover, brain infections may be recovered from animals that have never shown visible spirochætes in the blood, such as guinea-pigs inoculated with a Senegal strain of *S. duttoni* (Schlossberger and Wichmann, 1929).

These brain infections are particularly resistant to the action of chemotherapeutical agents, and therefore are very suitable for testing the efficacy of new spirochæticidal compounds. Whether the spirochætes are neurotropic and definitely attracted to the brain tissue, or merely persist there because, owing to the feeble circulation, they are not exposed to the action of a sufficient concentration of antibodies, is still a subject of dispute.

THERAPEUTIC USE OF RELAPSING-FEVER INFECTIONS.

The use of relapsing fever, instead of malaria, in the treatment of general paralysis was introduced by Plaut and Steiner (1919). Numerous authors have recorded results obtained by it, which in the main resemble those following the more usual treatment with malaria. If it is desired to employ relapsing fever for this purpose it is important to use a strain which is not resistant to arsenic, and since most of the laboratory strains of *S. duttoni* in Europe have acquired this property, some other strain is to be preferred. Sagel (1928) has obtained good results with a strain of Spanish relapsing fever, but it was found necessary to use either cultures or infected blood for inoculation into patients, as the bites of infected ticks produced too severe attacks. Although strains of relapsing fever are more easily maintained in the laboratory, it is doubtful whether their use presents any advantages over malaria treatment, and if the strains are kept in rodents contamination with rat-bite fever is another possible source of danger.

TREATMENT.

Relapsing fevers, in general, may be treated with salvarsan or one of its many derivatives. As a rule this treatment gives satisfactory results, except in under-nourished patients, who are very liable to succumb to the infection. It should be noted that, as in the case of trypanosomes, relapsing-fever spirochætes are liable to become arsenic-resistant and at present most of the strains of *S. duttoni* in Europe seem to have acquired this property. Infections with such strains may be treated by compounds such as sodium potassium bismuth tartrate, which J. Todd (1930) recommends for the general treatment of relapsing fevers, as it may be administered intramuscularly and is much less expensive than salvarsan or novarsenobenzol. The dose for an adult is two intramuscular injections on two successive days of 0.2 gm. of the drug dissolved in 2 c.cm. sterile water.

In addition to chemotherapeutic agents, convalescent serum has been used and is stated by Hoffmann (1921) to be as efficient as salvarsan, on condition that the serum is collected from a patient 2 to 10 weeks after an attack of the disease.

. METHOD OF TRANSMISSION.

In nature, all known varieties of relapsing fever are transmitted exclusively by the agency of lice, ticks, and possibly of bed-bugs. In many cases, infection is not the direct result of the bites of these arthropods but is produced indirectly by contamination of the surface of the body with infective material.

This process is essentially the same in the case of lice, where it is necessary to rupture the body of the insect in some way in order to liberate the infective contents, and in ticks, which pass out infective material in their fæces and coxal secretions. This infective material may enter the body either by the wound caused by the bite of the arthropod, or be introduced as a result of scratching the site of the wound. Different kinds of arthropods have become adapted to the transmission of the various strains of relapsing fever as indicated in the summary dealing with the main characteristics of these strains (see p. 164).

Although this summary indicates the usual transmitting host, in many instances other insects and ticks have been shown to be capable of acting as alternative hosts. Thus Manteufel (1908) and Neumann (1909^a) were able to transmit the European and American strains of relapsing fever by means of *O. moubata*, although both are normally transmitted by lice. Also the European and African strains can be transmitted from rat to rat by the agency of the rat-louse, *Hæmatopinus spinulosus*. The Spanish relapsing fever normally carried by *O. maroccanus* can also be transmitted by *O. moubata* and *Hæmaphysalis inermis* (Brumpt, 1926) and by *O. savignyi* (Nicolle and Anderson, 1926).

The details of the development of the spirochæte in the invertebrate host will be considered in the two most familiar examples, viz. *Ornithodoros moubata* and *Pediculus humanus*.

Transmission of Spirochætes by O. moubata.

The transmission of African tick fever by *Ornithodoros moubata* was first demonstrated by Dutton and Todd (1905), and the ease with which these ticks may be transported and reproduce the infection in laboratory animals is doubtless responsible for the familiarity of this strain. When these ticks are fed on infected blood, either human or animal, they become infected and are able to transmit the disease when fed on other vertebrate hosts. As a rule infection is not produced by the bite, as shown by Leishman (1910), who found that when infected ticks were removed from a monkey, before they had secreted any coxal fluid, the animal remained healthy.

By inoculation experiments it has been proved that the fæces commonly present on the under-surface of a feeding tick are infective, and the coxal secretion, which is generally poured out by the tick before leaving its host, mixes with these fæces and may enter the wound caused by the tick's bite. This contaminative method of infection seems to be the rule with *Ornithodoros moubata*, but in some other ticks, e.g. *Argas persicus*, the carrier of *S. anserina*, the infection may be introduced by the bite itself, as spirochætes can be found in the salivary ducts (Marchoux and Couvy, 1913).

Once having acquired the infection, *Ornithodoros* may remain infective throughout life, and in such cases the infection is transmitted to the offspring. This hereditary transmission in the intermediate host was first noticed by Dutton and Todd (1905), and subsequent experiments have shown that such infections persist indefinitely in *O. moubata*, for ticks several generations after an infective feed have been found to contain spirochætes. These observations suggest that the tick is the natural host of these organisms, which have no obvious harmful effects on it.

Some ticks seem to be resistant to infection, for in a series of transmission experiments with *O. moubata*, the writer (Hindle, 1911) found 5 uninfected out of 19 individuals that had all fed on the same heavily infected animal. Schuberg and Manteufel (1910) also found that ticks fed on an infected mouse and kept at 22° C. became non-infective after a few months and could not be reinfected. Moreover, after feeding on an infected animal the gut contents of these ticks only remained infective for two days.

The course of development of the spirochætes in ticks is still unsettled, as different observers have obtained conflicting results. Dutton and Todd (1907) noted the rapid disappearance of spirochætes from the tick's gut and their breaking up into granules, and suggested that these might represent a method of reproduction. Leishman, however, was the first to follow up this point of view, and in a series of papers from 1909 onwards described stages in the development of granules into spirochætes.

When an *Ornithodoros* ingests blood containing *S. duttoni*, the spirochætes pass through the wall of the alimentary canal (Fig. 28) and appear in the coelomic fluid; they are thus brought in contact with all the organs

in the body and may be observed to penetrate into the tissues. The spirochætes in the tick may remain motile for several days, but ultimately disappear when the animal is kept at moderate temperatures. Some of the spirochætes lose their motility, collect in tangled masses, and ultimately break up into fragments. These are supposed to be the chromatin granules found in the Malpighian tubules and other organs of the tick. Other spirochætes develop a lateral or terminal bud containing three or four highly refractile granules and these buds correspond in appearance to isolated granule clumps.

When ticks from which spirochætes had disappeared were kept at a higher temperature (about 30° C.), short forms, 3 to 4 μ in length, and showing only two or three curves, made their appearance. In the following days typical spirochætes appeared. In several such experiments these spirochætes again disappeared, to be followed by a new crop after six or seven days, suggesting the existence of a regular relapse in the tick analogous to what occurs in man.

The granules are almost constantly found in various tissues of *Ornithodoros*, and the inoculation of tissues containing such granules, but, as far as could be determined, no spirochætes, frequently resulted in the production of spirochætosis in mice. The occurrence of similar granules in the eggs of female ticks and the almost invariable absence of spirochætes from eggs, also suggests the possibility of these granules being the stage in which the infection is passed to the next generation of ticks (Leishman, 1920).

Hindle (1911), by the inoculation of various organs of infected *Ornithodoros* into mice, obtained results confirming the view that infection might be produced by the inoculation of tissues in which spirochætes could not be detected by ordinary microscopical examination. On the other hand, Koch (1906) and Carter (1907) found spirochætes in the eggs laid by infected ticks, but in both cases these were kept at comparatively high temperatures.

Wittrock (1913) stated that he was able to find granules in uninfected as well as infected *Ornithodoros*, and came to the conclusion that there was a continuous but slow multiplication of spirochætes in the tick at low temperatures. Kleine and Eckard (1913) found that spirochætes were continually present in infected ticks, the parasites occurring most frequently in the ovaries, and in descending order in the coxal glands, Malpighian tubules, cephalic glands, stomach and salivary glands.

It is difficult to reconcile these conflicting views, but the influence of temperature on the development in the tick seems to have been neglected in the case of the last four publications. The presence of granules in normal ticks apparently indistinguishable from those derived from spirochætes is a difficult obstacle to the study of these organisms in the intermediate host, but the available evidence shows that visible spirochætes do disappear from the stomach and intestine, and only later become recognizable in the tissues. It is difficult to accept the view that

in a host so well adapted for carrying the infection, the conditions in the gut should be so unfavourable that all except one or two particularly resistant individuals are killed off when the tick ingests infected blood. Yet some such hypothesis is necessary if it is assumed that the disappearance of spirochætes is only apparent (i.e. due to their being present in very small numbers) and not real.

The author, therefore, inclines to the view that spirochætes, after being ingested by the tick, divide up into very short granular forms, resulting in their apparent disappearance from the tick for at least some days, but further work on this question is much to be desired.

Transmission of Spirochætes by Pediculus humanus.

Nicolle, Blaizot and Conseil (1913) were the first investigators to succeed in proving experimentally the transmission of relapsing fever by lice, although previous observations, especially those of Mackie (1907) and Sergent and Foley (1908 and 1910) had indicated the extreme likelihood of these insects being the intermediate host. The first-named authors used an Algerian strain of relapsing fever, and were able to show that whereas the bite of an infected louse is innocuous, the body contents of such an insect are highly infective, and, if applied to a mucous surface or an excoriated surface, produce infection. In nature, lice are often injured, for their legs readily break off and allow the escape of the infected coelomic fluid. Also, when a man is bitten by a louse the irritation produced often causes him to scratch that part of the skin and thus the insect is often crushed on to the skin and its contents get on to the finger-nails. The excoriation of the skin produced by the scratching is sufficient to allow the entry of the spirochætes present in the body of the louse and thus infection occurs. In one experiment, a human patient was bitten between 30,000 and 40,000 times by infected lice without becoming infected, and yet when the contents of a single infected louse were introduced into the conjunctival sac, the patient developed an attack of relapsing fever.

When the spirochætes are ingested by lice kept at 28° C. they disappear entirely after one day, though some of the organisms may be seen penetrating the cells lining the stomach and oesophagus. What happens during the disappearance of spirochætes has not been followed, for owing to the presence of granules in the cells of normal lice it is difficult to trace any development. After a negative interval of six or more days, spirochætes reappear in the louse and are found in the body cavity. They increase in number in the coelomic fluid and then persist indefinitely. Occasionally they invade the eggs of the female and thus the infection may be transmitted to the offspring of infected lice, but such hereditary transmission is exceptional.

According to Nicolle and his fellow-workers the spirochætes have three stages in the louse: (a) indifferent granules, (b) an invisible and highly infective stage, and finally (c) the adult spirochæte. Evidence in support of the existence of this invisible stage is afforded by the results

of transmission experiments with Spanish relapsing fever. Although this strain is transmitted by lice, the spirochætes, after disappearing from the insect shortly after ingestion, never reappear as in the case of ordinary relapsing fever, although the contents can be shown to be infective.

Although Toyoda (1913), working with European and American relapsing fever, claims to have found unchanged spirochætes in the coelomic fluid of lice from the second to the seventh day after an infective feed, Nicolle's results have been confirmed by Chapcheff (1925), and the present evidence supports the view that spirochætes, as such, soon disappear after being ingested by the louse, but often reappear after a negative interval of about a week. The state in which they persist during the negative phase is still an open question.

In addition to the body-louse, bed-bugs have often been supposed to be the carriers of relapsing fever, and Karlinski (1902), Mackie (1907), Wenyon (see G. U. Smith, 1909), Nuttall (1908¹ & ²) and Dunn (1923) have shown that the spirochætes can survive in the gut of this insect for some weeks. Also, by taking bugs that had fed on a heavily infected animal and immediately allowing them to feed on a healthy one, direct transmission was sometimes obtained. Rosenholz (1927) found that when spirochætes were ingested by bugs the organisms invaded the hæmocœle and persisted in this fluid indefinitely, although they gradually disappeared from the gut. These spirochætes in the hæmocœle retained their virulence, and presumably could reproduce the infection in human beings in the same way as the body-louse, by the infected contents getting on to an excoriated surface, for the mere bites of such infected bed-bugs were quite innocuous.

It will be evident that in the case of relapsing fevers different races have become adapted to transmission by various species of ticks and biting insects, and in some cases certain infections seem to be transmitted exclusively by particular species. In most instances, however, further investigations have shown that practically all strains of relapsing fever may survive in a large variety of arthropods, which under favourable conditions may serve as the transmitting host. These conditions vary in different countries, and so we find that where human ticks are prevalent the local relapsing fevers are generally transmitted by these parasites, whilst when they are absent the body-louse is the usual carrier, although the bed-bug may also serve as a reservoir.

In addition to the usual method of transmission by the agency of specific intermediate hosts, it is possible for relapsing fever to be transmitted when any blood-sucking insect passes from biting an infected person almost immediately to another subject. If its proboscis is still contaminated with infected blood the infection may be introduced directly when the insect bites the new host. In this way Schuberg and Kuhn (1911) succeeded in transmitting *S. recurrentis* and *S. duttoni* by the bites of *Stomoxys calcitrans*.

The spirochætes are well known to be able to pass through the intact skin and mucous membrane, as shown by the experiments of Mackie (1907), Manteufel (1908), Nattan-Larrier (1909), Gózóny (1911) and many others. Consequently it is possible for relapsing fever to be acquired by simple contamination of the skin with blood containing spirochætes, and certain laboratory infections seem to have been acquired in this way (Gray, 1928).

ANIMAL RESERVOIRS.

Nicolle and Anderson (1927), mainly as a result of their extensive observations on relapsing fever in Tunis, have developed the interesting hypothesis that blood spirochætes commenced as parasites of small mammals, and that rodents commonly serve as animal reservoirs of the disease.

According to their view, relapsing-fever spirochætes start as an infection of small burrowing mammals, which is transmitted by the agency of ectoparasites, such as various species of *Ornithodorus*. These ticks are commonly found living in the burrows of rodents and thus have the opportunity of becoming infected by feeding on the blood of these mammals. The adult stages of these ticks commonly feed on larger mammals and thus the virus has a chance of being inoculated into them. In this way human beings may become infected by the ticks, at first probably more as an occasional accident.

When the virus has become adapted for living in human beings the next stage is supposed to be the adaptation of the spirochæte for living in the louse, although it is still transmissible by the tick. Finally, the spirochætes become completely adapted to the louse. Thus, from Africa, where ticks are common, they have spread to other parts of the world where transmission is effected solely by the agency of lice.

In support of their hypothesis the authors have shown that almost any strain of relapsing-fever spirochæte may be transmitted by the various species of *Ornithodorus*. In order to effect this transmission it is advisable to use the nymphal stages of the ticks, as the adults, although becoming infected, rarely become capable of transmitting the infection by their bite; also it is necessary that the blood of the vertebrate host should contain large numbers of spirochætes. Thus *Ornithodorus moubata*, in addition to tick fever (*S. duttoni*), has been shown capable of transmitting *S. hispanica*, *S. marocana*, *S. sogdiana* and *S. normandi*; *O. savignyi* can also transmit all these spirochætes; *O. erraticus*, all, with the possible exception of *S. normandi*; *O. normandi* transmits *S. normandi*, *S. duttoni* and *S. sogdiana*.

Whenever an experiment has been continued long enough, hereditary transmission was observed in the ticks, and this transmission is capable of passing through several generations when the tick is the natural carrier, but disappears sooner when the tick is a strange host. For example, *S. hispanica* in *O. moubata* will only pass through three generations, whilst *S. duttoni* in the same species of tick seems to persist indefinitely.

Any attempt to transmit blood spirochætes by the agency of genera of ticks other than their natural hosts, or by other invertebrate carriers, meets with greater difficulties, and is only partially successful, as in the case of *S. anserina* in *O. erraticus*, and *S. hispanica* and *S. duttoni* in the louse.

The relation between any particular spirochæte and the tick by which it is transmitted in nature is evidently only a geographical one, and consequently the habits of these ticks is the only check on the spread of the infections they transmit.

Varieties of Human Relapsing Fever.

At the present time there is no general agreement as to which of the races of blood spirochætes should be regarded as distinct, and any arrangement into groups is quite arbitrary. The following is a list of the better-known varieties in which we have followed Mühlens (1930) in arranging them according to the countries in which they occur.

EUROPE.

European Relapsing Fever (*S. recurrentis* Lebert, 1874).

This was the first relapsing-fever spirochæte to be described, and was observed by Obermeier in 1868, who did not publish his observations, however, until 1873. Münch, in Moscow, and Vandyke Carter (1882), in India, by inoculation experiments with volunteers, established the fact that it is pathogenic and produces a relapsing type of fever. Lebert (1874) named the organism *Protomycetum recurrentis*, and the following year Cohn (1875) named it *Spirillum obermeieri*. Consequently the specific name *recurrentis* has priority and should supersede the more generally used *S. obermeieri*.

The European relapsing fever due to this parasite is especially prevalent in Russia and Eastern Europe, and during the War epidemics occurred in many parts of the Eastern front. The disease generally accompanies wars and famines, and under these conditions has a much higher mortality than the usual 3 to 5 per cent.

The infection is transmitted by means of lice, and particulars of this method of transmission are given on p. 161. It is possible that the bed-bug may play some role in transmission, but the epidemiological evidence shows that it can only be of very secondary importance. It is true that Nuttall (1908¹) showed that the bed-bug was capable of transmitting the infection mechanically, and recent work has demonstrated that the spirochætes remain alive in its body cavity in the same way as in the louse (Rosenholz, 1927). In the case of both insects, however, the bite is innocuous except immediately after feeding, and it is necessary to rupture the insect and liberate the infective contents on the surface of the skin. This is very liable to happen in the case of a parasite like the louse, which remains in the clothes or on the body, but not in the bed-bug, which is rarely killed on the surface of the body and generally escapes after feeding.

The disease in man commonly shows three febrile attacks, the first of six days, the next of four days, and the third of three days' average duration, separated by apyretic intervals of approximately a week. Two more febrile attacks are sometimes observed, making five in all, any such later attacks being shorter and milder. The mortality of the disease varies considerably according to the general condition of the patient, but in under-nourished persons is very high. Moreover, any complications such as infection with paratyphoid may increase the mortality rate up to 50 per cent. As a general rule, however, the mortality is in the neighbourhood of 3 to 5 per cent.

The monkey is the only laboratory animal that can easily be infected directly from human cases of this disease. After passage through monkeys the infection can be maintained in rats and mice. After the inoculation of a large number of spirochætes directly from a human patient into mice, the organisms may appear in the circulation for one to two days, but there are no relapses. Guinea-pigs and rabbits are resistant.

Spanish Relapsing Fever (S. hispanica de Buen, 1926).

In *Spain* a tick-transmitted variety of relapsing fever has been investigated by Sadi de Buen (1926), who gave the name *Spirochæta hispanica* to the causative organism. This infection is transmitted by *Ornithodoros maroccanus*, and according to Nicolle and Anderson (1928) is identical with the tick-transmitted relapsing fevers of Morocco (see below, p. 168).

S. hispanica is much more pathogenic to laboratory animals than the ordinary *S. recurrentis*, and, in addition to monkeys, mice, rats, rabbits, and especially guinea-pigs, can readily be infected. The clinical symptoms of the disease are intermediate between those of European relapsing fever and African tick fever, and the infection can readily be treated with neosalvarsan.

ASIA.

The majority of Asiatic relapsing fevers resemble the European form in their general characters, and most of them seem to be transmitted by lice, although the Central Asiatic group are transmitted by various species of *Ornithodoros*.

Indian Relapsing Fever (S. carteri Mackie, 1907).

The best-known variety is the Indian relapsing fever, *S. carteri*, first described by Carter (1877), and subsequently studied by many observers, including Mackie (1907, 1920), Steen and Townsend (1913), Smith and Graham (1913), Gill (1922), Cragg (1922), Russell, Ayyar and Ubhaya (1924) and Cunningham (1925). This disease is very widespread in India and has been recorded from the Himalayas to the extreme South. Although the number of febrile attacks is comparatively few, three-quarters of the cases showing only one or more often two attacks, the mortality may be extraordinarily high, Mackie recording one of 38 per cent. out of 3,196 cases

in Bombay. Malnutrition is doubtless a contributory factor to such high mortalities, but in general the prostration after the first attack is much more severe in the case of Indian relapsing fever than in the European variety.

The infection is transmitted by the agency of lice, as shown by Mackie (1907) in his pioneer experiments on this subject. Brown (1913) made some interesting observations on an epidemic in the Meerut district and showed that there was a marked rise in the incidence of the disease in May and December, especially affecting the males, and this increase corresponded with the gathering of the crops, when the villagers congregated on the threshing-floors. As this observer remarks, it is difficult to explain the incidence of the disease on the assumption that the louse is the only carrier, although the fact that the agricultural labourers on any particular farm pile their clothes in a heap would favour the distribution of lice. Very large numbers of the immature stages of a Pentatomid bug, *Bragada picta*, were observed biting the occupants of the threshing-floors, which included both diseased and healthy persons, and it is possible that under such conditions mechanical transmission of the disease might occur. The spirochæte of Indian relapsing fever, *S. carteri*, is only feebly pathogenic to laboratory animals, but monkeys, rats, mice and squirrels may be infected directly from human cases. Monkeys generally show a single relapse after the first attack (Cunningham, 1925), but the spirochætes are often extremely scanty during this relapse and can easily be overlooked.

Quetta Relapsing Fever.

Browse (1912) has brought forward epidemiological evidence in support of the view that Quetta relapsing fever is transmitted by ticks, either *Argas persicus*, or possibly *Ornithodoros tholozani*. It seems to resemble African tick fever in the number of febrile attacks, for in contrast with the usual one or two relapses of ordinary Indian relapsing fever, some of the patients showed six or seven.

Persian Relapsing Fever (S. persica Dschunkowsky, 1913).

The existence of relapsing fever in Persia had been suspected for many years, but Dschunkowsky (1913) was the first to bring forward any definite evidence on the subject. He received a consignment of ticks from that country, which, when fed on a human subject, produced an attack of relapsing fever with three to four relapses. There is some doubt as to the ticks producing these infections, as, although they were identified as *Ornithodoros tholozani* or *O. canestrinii*, the figure of the tick accompanying the paper recalls *O. lahorensis*. Wright and Harold (1920) record having found *O. lahorensis* and *Argas persicus* in the bed of a patient suffering from relapsing fever, and in the absence of lice considered that the ticks were responsible for the transmission. Other authors have described cases of relapsing fever associated with the presence of ticks, but it must be admitted that the evidence incriminating *Argas persicus* as the carrier

of this disease, known locally as ' Miana ', is very unsatisfactory. Probably the local species of *Ornithodoros* have been confused with *Argas*, for up to the present this fowl tick has never been shown to be the natural carrier of any human infection.

Bokharan Relapsing Fever (*S. usbekistanica* Pickoul, 1928).

An epidemic of relapsing fever transmitted by ticks was observed by Pickoul (1928) at Fergan, in Bokhara. The infection may be transmitted to rats, mice and guinea-pigs, the latter being very susceptible to the disease and showing a mortality of about 40 per cent.

These varieties all belong to the Central Asiatic group of relapsing fevers, which, as a general rule, seem to be transmitted by the agency of ticks, either *Ornithodoros tholozani*, *O. lahorensis* or *O. papillipes*. It is possible that lice may also play some part in the spread of the infections, but hitherto the only definite evidence incriminates ticks.

In addition to the above, Nicolle and Anderson (1928) produced a spirochætal infection in guinea-pigs with the bites of *Ornithodoros papillipes* received from Bokhara. This infection can be transmitted to man and may represent another local variety of relapsing fever. It differs from other strains in the type of infection produced in guinea-pigs and has been named *S. sogdiana*.

Relapsing Fever in Asia Minor.

In Asia Minor relapsing fever has been recorded from Palestine (Cropper, 1905) and from Syria to Iraq. As a general rule lice seem to be the usual transmission agents (see Mühlens, 1930) although *Argas persicus* has been suggested as the probable carrier of the disease in Palestine (Nicholson, 1919). It is quite possible that other species of ticks may have produced the infections recorded by this observer, as it is very easy to overlook the presence of *Ornithodoros*.

A variety of relapsing fever has also been recorded from Southern Arabia (R. M. Carter, 1908) which is said to be identical with African tick fever and also to be transmitted by ticks.

Relapsing Fever in Eastern Asia.

In Eastern Asia various kinds of relapsing fever have been recorded which, in their general characters, closely resemble the ordinary European variety, and seem to be transmitted by the agency of lice and possibly bed-bugs.

Chinese relapsing fever was first noted by Hill (1904) in Pakhoi, and it has subsequently been recorded from most provinces in China. The infection may be transmitted to the usual laboratory animals and has been studied among others by Meleney (1927), who was able to produce intense infections in splenectomized squirrels.

Manchurian relapsing fever has been described as a distinct variety by Toyoda (1919), and similar epidemics have been recorded from Japan. In *Indo-China* the so-called *Tonkin relapsing fever* was first noticed by

Seguin in 1907, and the spirochæte studied in animals by Mathis and Leger (1911¹). Monkeys and white mice can be infected directly from human cases, and after passage through mice the virulence of the parasite increases. According to Mouzels (1913) any blood-sucking insect, and especially *Culex*, may spread the infection, but this seems doubtful in the absence of any experimental evidence other than the persistence of the spirochætes in the gut of these insects, and the disease is probably another of the louse-borne relapsing fevers occurring throughout the whole of Eastern Asia.

AFRICA.

North African Relapsing Fevers.

The African relapsing fevers constitute a very large group, some of which are transmitted by ticks and others by lice. The North African relapsing fevers have been very extensively studied in Tunis by C. Nicolle and his fellow-workers, and in Algeria by the Sergents, and our knowledge of the relationships of the various strains in that region is probably more complete than in any other part of the world.

The most widespread strain is a louse-borne variety extending along the Mediterranean from Morocco to Egypt and southwards into the Sudan. The spirochæte of the Algerian relapsing fever has been named *Spirochæta berbera* (Sergent and Foley, 1910) and that of the Sudanese strain *S. ægyptica*. These strains all agree in producing a type of relapsing fever resembling the European variety in the number of febrile attacks and the general mortality. Moreover, the spirochæte has little pathogenicity towards laboratory animals. Monkeys can be infected by direct inoculation from human cases, but the strain cannot be maintained in these animals, and rats and mice are even more resistant (Sergent and Foley, 1910).

This North African spirochæte was used by Nicolle, Blaizot and Conseil (1913) in their experiments demonstrating the exact mechanism of louse transmission (see p. 161).

In addition to this louse-borne type of relapsing fever there are a number of North African strains transmitted by ticks.

The spirochæte of *Moroccan relapsing fever* (*S. hispanica* var. *marocana*) seems to be practically identical with the Spanish strain (*S. hispanica*). It was first recorded by Breeze (1909) and has been studied in particular by Nicolle and Anderson (1928, 1929), Nicolle, Anderson and Hornus (1929), and Delanoë (1929). The infection is transmitted by *Ornithodoros maroccanus*, a tick which may be found in the burrows of various wild mammals, including the smaller desert rodents, the porcupine, fox and badger. It also occurs in the neighbourhood of pig farms. The ticks collected from the burrows of animals in the hills away from human habitations may be found infected with spirochætosis, a fact which supports Nicolle's hypothesis that this infection is primarily one of the smaller burrowing rodents. The observations of Nicolle, Anderson and

Hornus (1929) show that these strains exhibit considerable variations in their immunological characters, to such an extent that in Morocco it is almost possible to distinguish a different 'species' from each locality. As these authors remark, it is more reasonable to consider the tick-transmitted relapsing fevers of Morocco and also Spanish relapsing fever as forming a natural group, in which the spirochætes show a very marked tendency to individualization.

In *Tunis* the local species of *Ornithodoros* are found infected with a number of different varieties of spirochætes such as *Spirochæta normandi* in *O. normandi*, *S. normandi* var. *carthaginensis* in *O. normandi* and *O. erraticus*, and there seems little doubt that these and other spirochætal infections of the natural rodents are communicable to human beings.

Further east in Abyssinia and Somaliland the local relapsing fevers may be transmitted by *Ornithodoros savignyi*, Brumpt (1908), Drake-Brockman (1915), or by *O. moubata*.

The Relapsing Fevers of Tropical Africa.

The relapsing fevers of Tropical Africa, like those of the North, include both louse-borne and tick-transmitted varieties, of which by far the best known is the so-called *African tick fever*.

African tick fever (*S. duttoni*). This variety of relapsing fever was first described as a spirochætal infection by Cook (1904) and P. H. Ross and Milne (1904), and the latter showed that the infection was carried by ticks. Independently Dutton and Todd (1905) in the Congo had also discovered the spirochæte in the blood of patients, and that it was conveyed by the bite of *Ornithodoros moubata*, and, in addition, made the very important observation that the infection was hereditary in the ticks. *O. moubata* can readily be transported, will live for long periods without food, and also can be reared in the laboratory without any difficulty, consequently the infection it conveys has been very extensively studied, and is the strain of relapsing fever maintained in most laboratories.

The disease produced by *S. duttoni*, as a general rule, is more severe and shows many more relapses than the European variety. The mortality in normal persons, however, rarely exceeds 5 per cent., but under-nourishment or other adverse conditions increase it considerably.

Ornithodoros moubata, the natural carrier of this disease, has been recorded from many parts of Africa from Abyssinia in the North to the Orange River in the South, and also from Madagascar. It has not yet been recorded on the West Coast further North than Angola. The human tick fever that it transmits has been recorded from most of these localities, although sometimes it is combined with a louse-borne type of relapsing fever. Practically all laboratory animals can readily be infected with *S. duttoni* by the inoculation of blood containing the spirochætes. The bites of infected ticks are a much less certain method of infection, except in the case of monkeys, which are very susceptible to the disease and generally succumb, no matter what method of transmission is used.

The tick-transmitted relapsing fevers of Tropical Africa, unlike those of the Mediterranean region, seem to constitute a very homogeneous natural group, and strains of *S. duttoni* from widely different localities have been found to show a close agreement in their immunological characters. Nevertheless, various names have been proposed for the tick-fever spirochætes from different localities, such as *S. kochi* (Novy, 1907) for the East African strain, *S. rossi* (Nuttall, 1908²), also for an East African strain, the term *S. duttoni* being reserved for the West African one (Novy and Knapp, 1906).

In addition a spirochæte which seems to be identical with *S. duttoni* (see Mathis, 1928) has been found in the shrew mouse *Crocidura stamplii*, at Dakar, Senegal. A. Leger (1917), who discovered the spirochæte, proposed the name *S. crociduræ* for this organism, by which name it has been known until recently. The local-strain spirochæte causing relapsing fever in man is identical with the shrew-mouse spirochæte and also with *S. duttoni*, but hitherto no *Ornithodoros* has been incriminated as the carrier in that region.

Epidemics of a louse-borne type of relapsing fever have been recorded from French West Africa (Nogue, 1925), the Gold Coast and Nigeria (McCulloch, 1925 ; Caffrey, 1926), and French Sudan (Kerrest, Gambier and Bouron, 1922), and since the War a great epidemic of this disease has swept across equatorial Africa, via Chad and Wadai, until it reached Darfur. Some idea of the magnitude and severity of this epidemic may be gathered from the fact that in one district of Darfur alone 10,000 died out of a total population of 45,000 (Atkey, 1929). This epidemic now seems to have died out except in isolated centres.

Selwyn-Clarke, Le Fanu and Ingram (1923) made a careful investigation of an epidemic in the Gold Coast, which in its general characteristics closely resembled the European type of relapsing fever. Monkeys and rats could be infected but did not relapse, and guinea-pigs and rabbits were refractory. A local pouched rat was found to be very susceptible to the infection. The disease was transmitted by lice, and these authors were able to confirm the fact that it is not conveyed by their bites nor by their excretions, but by the inoculation of wounds by crushing the lice on the skin.

There seems little doubt that, in general, the West African group of relapsing fevers is more closely related to the European type than to the *duttoni* group, with the marked exception of the Dakar strain, which seems to belong to the latter.

AMERICA.

North American Relapsing Fever (S. novyi Schellack, 1907).

In North America, cases of relapsing fever have been recorded from various parts of the United States, but the disease is so rare as to suggest either that the cases are introduced or that man is not the usual host of the spirochæte. The infection shows a general resemblance to the

European variety, and the spirochæte after passage through monkeys can be transmitted to rats and mice. After 18 months to 2 years' passage in these animals it was still pathogenic to man (Mühlens, 1930). The causative organism has been named *Spirochæta novyi* (Schellack, 1907), and various authors have considered it distinct from the other varieties.

The method of transmission is uncertain, for, although lice seem the most probable agents in the majority of cases, Briggs (1922) has brought forward indirect evidence suggesting that two cases observed in California had acquired the infection as a result of tick bites, and suggested that *Ornithodoros* might be the transmitting host, since it is known to occur there. Hitherto, however, no definite evidence on the subject has been obtained.

South American Relapsing Fevers (*S. venezuelensis* Brumpt, 1921 and *S. neotropicæ* St. John and Bates, 1922).

In Central and South America, epidemics of relapsing fever have been recorded from Cuba, Mexico, the Panama region, Colombia, Venezuela, Peru, Uruguay and the Argentine. The spirochæte has been named *S. venezuelensis* in Venezuela and Colombia, and *S. neotropicæ* in Panama, and in its immunological reactions seems to differ from the European and African strains (St. John and Bates, 1922). The disease is generally transmitted by various species of *Ornithodoros*, although in Peru, del Prado (1920) considers that the louse is the sole carrier of the infection. Different species of *Ornithodoros* have been found to be the carriers in the various localities and probably any of those that bite man may become infected. In the Panama zone, Bates, Dunn and St. John (1921) have shown that *O. talaje* is the usual carrier. In Colombia and Venezuela, *O. talaje*, *O. venezuelensis* and *O. turicata* have all been incriminated, but *O. venezuelensis* seems to be the most important carrier (Dunn, 1927).

In addition to these ordinary types of relapsing fever pathogenic to monkeys and rodents, a Brazilian strain studied by Brumpt, Bourrol and Guimarães (see Brumpt, 1927) was found to be non-pathogenic to monkeys, rats and mice. Further observations on this strain are much to be desired.

AUSTRALASIA.

In marked contrast with all other parts of the world, relapsing fever seems to be unknown in the continent of Australia and hitherto the only record of the disease from that region is a single case recorded by Morin and Genevray (1926) from New Caledonia. Possibly this was introduced.

Blood Spirochætes Occurring in Animals.

Spirochætes have been recorded from the blood of a considerable number of animals, but in very few instances is any more information available than a brief statement as to the locality of the host, and occasionally the dimensions of the spirochæte. In many cases these so-called blood spirochætes are undoubtedly intestinal forms which have

become mixed with the blood as a result of perforation of the alimentary canal. This is especially the case in those from game animals, as their blood films are often contaminated with gut contents. The following is a list of some of the blood spirochætes which have been recorded from animals.

BLOOD SPIROCHÆTES OCCURRING IN MAMMALS.

Primates.

Under the name of *S. pitheci*, Thiroux and Dufougeré (1910) described a spirochæte found in the blood of a *Cercopithecus patas* from the French Sudan; they considered the organism to be related to *S. duttoni*, which it resembles in its morphology. The monkey from which it was obtained showed meningeal symptoms, and atrophy of the right eye, with corneal opacity. Rats, mice and field-mice, inoculated with *S. pitheci*, showed only a slight infection with enlargement of the spleen. After passage through mice the strain produced a fatal infection in another monkey, *C. patas*.

Spirochætes have also been recorded from a *Cercopithecus sabæus*, living in the London Zoological Gardens (Plimmer, 1912); and from *Cercopithecus ruber*, in the Lado (Ranken, 1912). Castellani and Chalmers (1910) recorded the presence of spirochætes in the blood of Ceylon monkeys (probably *Macacus sinicus*) and gave the name *Spirochæta macaci* to the organism, and in addition Balfour and Leishman have observed spirochætes in various species of monkeys.

Chiroptera (Bats).

Nicolle and Comte (1906) were the first to describe a spirochæte occurring in the blood of a bat in Tunis. This organism, *Spirochæta vespertilionis*, was studied by Gonder (1907); in addition to *Vespertilio kuhli* it also occurs in *Vesperugo pipistrellus* in England (Coles, 1914). These spirochætes are 12 to 18 μ long by 0.25 μ in diameter, with pointed extremities, and during the initial attack are very numerous in the blood. The organism has been observed in the alimentary canal of ticks feeding on infected bats, and probably either *Ixodes* or *Argas vespertilionis* are responsible for its transmission.

Carnivora.

Spirochæta canina Bosselut (1925) was observed in the blood of a dog at Marengo, Algeria. It is 8 to 22 μ in length and 0.3 μ in diameter, with 3 to 6 spirals, often irregular. Other species have also been recorded, such as the common *S. regaudi* in the acid cells of the stomach, spirochætes resembling *S. recurrentis* in ulcerous lesions of dogs in India (James, 1905), and in addition a leptospira. Sambon also observed a spirochæte in the gastric ulcer of a fox.

Spirochæta lutræ Prowazek (1907) was observed in the blood of an otter. It is said to be large, with 3 to 6 spirals, and to contain four chromatic granules; the extremities are rounded. The nature of this organism is very doubtful.

Insectivora.

Spirochæta crociduræ, now considered identical with *S. duttoni*, was discovered by A. Leger (1917) in a shrew mouse, *Crocidura stamptii*, at Dakar, Senegal. The natural occurrence of a spirochæte of human relapsing fever in such a host is of considerable interest, for it indicates one of the ways in which infection may be maintained in the absence of any human host.

Rodentia.

Spirochæta raillieti Mathis and Leger (1911²) was found in the blood of a rabbit at Tonkin, the only one out of 200 examined that was infected. The majority of the organisms were 14 to 17 μ in length, 0.25 μ in breadth, and showed 4 or 5 spirals. This spirochæte seems to be non-pathogenic.

Spirochæta naganophila E. Savini (1923) is a doubtful species which appeared in the blood of mice inoculated with a strain of *Trypanosoma brucei* (Savini, 1923). It is 10 to 20 μ in length, with an average of 15 to 16 μ , and approximately 0.3 μ in thickness. There are usually about 10 spiral turns, which are rounded and rather irregular. The organism is difficult to stain, and in life is extremely motile. According to Savini (1923), it was also present in the intestine, and it is suggested that the trypanosome infection favours the invasion of the spirochæte into the blood. The spirochæte is said to be non-pathogenic.

Vinzent (1927) obtained a similar organism in white mice infected with a strain of *Trypanosoma somaliense*. The spirochæte was isolated and maintained its virulence for 50 days when left in Illert's medium. It was found to be transmissible to young rats and mice, in which it produced a great increase in the number of mononuclears. In addition, the organism was inoculated into a woman suffering from paralysis, and appeared in the blood on the sixth day accompanied by a rise in temperature.

It is difficult to decide the nature of these two records of mouse spirochætes. The chance of contamination is always present when different strains are maintained in laboratories, and the fact that in both instances the spirochætes were found in mice inoculated with blood containing trypanosomes is suggestive of such an occurrence.

Spirochæta normandi Nicolle, Anderson and Colas-Belcour (1927) was first observed in the contents of ticks, *Ornithodoros normandi*, inhabiting the burrows of a gerbil, *Meriones shawi*, at Kef, Tunis. Laboratory experiments, as well as field observations, showed that this rodent is the vertebrate host of the spirochæte. The white mouse is also very susceptible, showing four or five attacks, but white rats are only slightly susceptible, and guinea-pigs refractory. A monkey was infected and showed four relapses. Attempts to transmit the disease by means of lice were negative. *Ornithodoros normandi* were found to transmit the infection by their bites, and to be infected in nature, so there can be no doubt that this species of tick is responsible for spreading the disease. The method of transmission, cross-immunity tests, and also the results

of animal inoculations, serve to distinguish *S. normandi* from the human relapsing-fever spirochætes, which it closely resembles. Nevertheless, it seems probable that relapsing fever due to this organism may be produced by the bites of *Ornithodoros normandi*, as this tick not infrequently bites human beings.

Spirochæta gundii Nicolle (1907) was found once in the blood of a North African rodent, *Ctenodactylus gundii*. It measures 16 to 19 μ in length by 0.3 μ in breadth. No pathogenic effects were observed in the host and the parasites disappeared after a few days.

Spirochæta caviæ Sangiorgi (1913) was observed by de Gasperi (1912), in cultures of the heart-blood of a guinea-pig inoculated with garden soil, but the author was never able to obtain the spirochæte in subsequent experiments. Sangiorgi (1913) found similar organisms in the blood films of guinea-pigs inoculated with plague material from rats. Macfie (1914) observed a spirochæte in a culture of heart-blood, containing glucose, of a guinea-pig at Lagos. This organism seems to differ from that of de Gasperi, as the majority were only 2 to 9 μ in length, with blunt extremities and 2 to 6 spiral turns. According to de Gasperi (1912) there are forms 16 to 24 μ in length by 0.3 to 0.4 μ in thickness, with five, six or eight spiral turns; and shorter forms, 9.6 to 12.8 μ in length, with three or four spiral turns. The extremities are pointed. Sangiorgi also distinguishes two types, a larger one, 9.6 to 11.2 μ in length, with 4 to 6 spiral turns, and smaller forms 3.2 to 4.8 μ in length by 0.3 to 0.4 μ in thickness, with 2 to 3 regular spiral turns.

There is no clear evidence as to the nature of any of these infections, and in view of the large numbers of spirochætes inhabiting the alimentary canal of guinea-pigs, it seems most probable that all the above records are examples of intestinal forms that have invaded the blood-stream. This is well known to occur in guinea-pigs under certain ill-defined conditions, the best example being the spiral organism described by Sanarelli (1927) under the name *Heliconema vincenti*. Although normally living in the cæcum, this organism may sometimes be isolated in pure culture from the heart-blood of guinea-pigs (see p. 117).

Spirochæta muris, first described by H. V. Carter (1887) in the blood of rats, *Mus decumanus*, in India, has subsequently been recorded from rats and mice in other parts of the world. Although there is some difference of opinion, most authors are agreed that this parasite is a *Spirillum* and not a spirochæte. Under the name of *Spirillum minus* it is described in Chapter VIII. The spirochætes observed by Lingard (1907) in the blood of a bandicoot, *Nesokia bandicota*, in India, and by Carpano (1913) in the blood of a marmot of Erythrea, are probably the same species.

Ungulata.

Spirochæta theileri Laveran (1903), described by Laveran from films supplied by Theiler, was originally found in the blood of cattle in the Transvaal. It measures 20 to 30 μ in length and closely resembles the

human relapsing-fever spirochætes. It has been repeatedly described from cattle in other parts of the world. Theiler (1905) and Dodd (1906) showed that it can be transmitted to cattle and sheep by the inoculation of infected blood. The horse spirochæte has also been similarly transmitted to cattle and sheep (Dodd, 1906). It is evident that the so-called *Spirochæta equi* (Novy and Knapp) of the horse, and *S. ovina* (Blanchard) of the sheep are identical with *S. theileri*, and probably other blood spirochætes described from ruminants all belong to the same species.

The inoculation of infected blood into a susceptible cattle is followed by an incubation period of two or three days. The spirochætes then appear in the blood for 13 to 17 days, but are generally few in number. After their disappearance the host is immune against reinfection, but its blood may remain infective to other animals for long periods after apparent recovery. Moreover, a secondary infection, such as piroplasmosis, may cause the reappearance of spirochætes in the blood long after apparent recovery.

The infection is transmitted in the Transvaal by *Margaropus decoloratus*, a one-host tick, which ingests the spirochætes and transmits the infection to its offspring; the larvæ are thus born infected. Brumpt (1919) showed that the Brazilian strain of this infection is transmitted by *Margaropus australis*. He also made the very interesting observation that some of these ticks failed to produce infection for seven generations and yet at the eighth generation infected a cow. This experiment shows how difficult it is to obtain uninfected ticks, for ticks that had been fed on cattle for seven generations without producing any signs of spirochætosis might reasonably have been considered free from infection. Experimentally, *S. theileri* can be transmitted by *Rhipicephalus evertsi*, a three-host tick, and in this species the infection is also hereditary (Theiler, 1909). *Margaropus calcaratus* is probably the carrier in Morocco, and in America Crawley (1916) has found spirochætes in *Margaropus annulatus*, which he considers identical with *S. theileri*.

Spirochæta suis. King, Baeslack and Hoffmann (1913), and also Bekensky (1916), have described spirochætes in the blood of pigs infected with hog cholera, but these are not of the relapsing-fever type. The organisms are 6 to 7μ in length by 1μ in thickness, and pass through a granule stage. They are supposed to enter the blood from the ulcers in the cæcum and to play some part in the production of hog cholera, but there is no definite evidence in support of this view. The organism is a strict anaerobe and can be cultivated in a mixture of horse-serum and pyrogallic solution.

In addition, Macfie (1916) found spirochætes in the blood of pigs on the Gold Coast, but these were probably derived from the intestine as in the foregoing records.

Blood spirochætes of other ruminants. Lingard (1907) found spirochætes in the blood of an Indian elephant, and also in the camel, but the latter record is doubtful as the organisms are said to have been inside the red

cells. Bruce, Hamerton, Bateman, Mackie and Lady Bruce (1911) described a typical spirochæte from the blood of an antelope, *Tragelaphus scriptus*, in Uganda. It measured 9 to 15 μ in length, and mice were successfully infected with it. Todd and Wolbach (1912) described a similar form from the blood of a roan antelope, *Hippotragus equinus*, in the Gambia. Lingard (1907) recorded the presence of spirochætes of the relapsing fever type in camels and elephants in India. Trautmann (Mühlens, 1913) and Mason (1916) have observed spirochætes in the blood of donkeys in Tanganyika Territory and Egypt respectively. These are probably identical with *S. theileri*, which includes those recorded from horses, sheep, zebras and cattle in various parts of the world.

Marsupialia.

Spirochæta didelphis Vianna, Figueiredo and Cruz (1912) was described from the blood of a Brazilian opossum, *Didelphis aurita*. This doubtful organism is said to have irregular spirals and to move slowly. It is inoculable into other opossums and white rats, and the spirochætes appear in the circulation 4 to 5 days after inoculation.

BLOOD SPIROCHÆTES OCCURRING IN BIRDS.

Spirochæta anserina Sakharoff, 1891 (Figs. 20 to 25).

This spirochæte was described by Sakharoff (1891) as occurring in the blood of sick geese in the Caucasus, and similar organisms were observed by Marchoux and Salimbeni (1903) in diseased fowls in South America, who also showed that the infection was transmitted by ticks, *Argas persicus*. Numerous specific names have been given to the different strains of spirochætes that have been recorded from fowls in most tropical and subtropical countries. For example, Nuttall proposed the name *S. marchouxi* for the South American strain, and independently Stephens and Christophers (1905) suggested the well-known name, *S. gallinarum*, for the same strain. Later, Brumpt (1909) separated strains from Senegal and Tunis under the names *S. neveuxi* and *S. nicollei*, respectively. Balfour (1911) suggested the name *S. granulosa penetrans* for the Sudanese form, and Neumann and Mayer (1914) gave the name *S. gallinarum* var. *hereditaria* to a North African strain studied by Hindle (1912²). Parrot (1920) observed similar organisms producing a fatal disease in ducks in Algeria, and suggested the name *S. anatis* for the spirochæte.

There is no satisfactory evidence in support of the specific distinctness of any of these strains, and the author agrees with Wenyon (1926) that the correct designation for all fowl spirochætes, including those of other domestic birds, such as ducks and geese, is *Spirochæta anserina*.

General account. The fowl spirochæte has been extensively studied in laboratories as it is readily transmitted by the inoculation of infected blood into fowls, geese, ducks, turkeys, Java sparrows, canaries and other small birds. Moreover, in the absence of birds the strain may be kept in *Argas persicus*, a tick which will live for many months without

food. As a general rule, fowl spirochætosis produces a high mortality, especially in young birds, but different strains vary considerably in this respect. In endemic regions the local fowls are often resistant, even when it is extremely difficult to obtain direct evidence of infection. Experimentally, it has been found possible to produce temporary infections in rabbits (Levaditi and Lange, 1905) and mice (Deutz, 1912). Fowls which have been exposed to infection generally show spirochætes in the blood after an incubation period of 2 to 5 days. In severe cases the organisms gradually increase in number until after two or three days the blood contains enormous numbers of spirochætes and the bird succumbs to the infection after four or five days, death generally being preceded by a crisis. Sometimes, after apparent recovery from the acute form, a chronic type of disease develops, with paralysis of the feet and wings, progressive emaciation and death in 8 to 15 days.

The spirochæte in the vertebrate host (Figs. 23 to 25). *S. anserina* closely resembles *S. recurrentis* in its general appearance, and also multiplies by transverse division. It may sometimes be observed to break up into coccoid bodies, as first described by Balfour (1911¹). This process commonly takes place when the spirochætes are under unfavourable conditions, such as after drug treatment, or at the crisis. Whether these bodies represent a phase in development or are merely degeneration forms is still undecided. In addition, large cyst-like swellings may also be developed, either at the extremity or along the length of the spirochæte, which seem to be the result of plasmolysis. Balfour (1908) drew attention to the presence of peculiar bodies in the red cells of Sudanese fowls, and found that they were associated with spirochætosis. The bodies were especially common in fowls suffering from the 'after-phase' or chronic type of infection. Balfour (1911¹) supposed that these intracorpuseular bodies represented a stage in the development of the spirochæte, but later (1914) suggested there were possibly two distinct infections, both transmitted by *Argas persicus*. Although the author suggested that these bodies might be the result of nuclear degeneration (Hindle, 1912²), subsequent experiments with fowls infected by means of ticks from the Sudan and Brazil, respectively, showed such marked differences that the occurrence of a mixed infection seemed a more probable explanation. Whereas fowls infected by the bites of Sudanese ticks often showed large numbers of intracorpuseular bodies, those infected by the Brazilian ticks never showed the same appearance, although rarely nuclear extrusions were present. Franchini (1924³), in Italy, has described similar bodies occurring in the blood of *Hypoleis hypoleis* without any associated spirochætal infection. Balfour (1911¹) described appearances in these intracorpuseular bodies suggesting that they originate as small granules which increase in size until they are almost the diameter of the nucleus of the red cell. The bodies then break up into a number of small granules by a kind of schizogony and the cycle is repeated. There is no direct evidence, however, connecting these bodies with spirochætes.

Recently, Carpano (1929) has shown that fowls imported into Egypt are liable to infection with a blood parasite *Ægyptianella pullorum*, and from his description there seems no doubt of its identity with these intracorpuseular bodies.

The spirochæte in the invertebrate host (Figs. 20 to 22). *S. anserina* is normally transmitted by the bites of infected ticks, of which three species, *Argas persicus*, *A. reflexus* and *A. miniatus*, are the most usual carriers, especially the first species. Marchoux and Salimbeni (1903), in South America, were the first to discover the method of transmission, using *A. miniatus*. Brumpt and Foley (1908) transmitted the disease by means of *A. persicus* collected in North Africa. *A. reflexus* was also shown by Schellack (1907) to be capable of carrying the infection. Hindle (1912^a), working with *Argas persicus* and a North African strain of spirochætes, first demonstrated that the infection will pass through the egg to the next generation and from this to the next one, the ticks being fed exclusively on normal fowls.

Experimentally, *Ornithodoros moubata* has been shown capable of transmitting the infection (Fülleborn and Mayer, 1908; Brumpt, 1908, and others). Mayer (1914) transmitted the infection from diseased to healthy canaries by means of mites, and in Austria, Gerlach (1925) has also succeeded by means of fowl mites, *Dermanyssus avium*, which are considered to be the chief carrier in that region.

The exact mechanism of transmission, including the development of spirochætes in the tick, has been the subject of much discussion. Although transmission can be effected by the bites of infected *Argas*, and Marchoux and Couvy (1913) have shown that spirochætes occur in the salivary ducts, Hindle (1912^a) is of the opinion that the contaminative method of infection is frequent. According to this view, the wound caused by the tick's bite is bathed with the infective fluid from the coxal glands, which was shown by him to be capable of reproducing the disease. Although most *Argas* pass out this secretion after having left the host, some of them secrete it whilst feeding, and these may be the cause of infection, for as a general rule it is necessary to feed several *Argas* on a fowl in order to produce spirochætosis.

Knowles (1929), in India, has shown that fowls may readily be infected by swallowing infected ticks, an observation that the author can confirm from experiments with *Argas* infected with a Sudanese strain of spirochætes. This is possibly the method of infection when the disease is transmitted by the agency of mites.

The life-history of the spirochæte in the tick has been studied mainly by Prowazek (1907), Balfour (1908), Hindle (1911 and 1912^a), Marchoux and Couvy (1913), and Knowles, Das Gupta and Basu (1929). It is doubtful whether Prowazek's account of spirochætes dividing longitudinally until they become so fine as to be invisible, has any supporters at the present time. Balfour and Hindle agree in the main outlines of the life-cycle, namely, that after reaching the gut of the tick, the spirochætes

bore their way through the gut-wall and invade the coelomic fluid, where they may be found within an hour after an infected meal. From the coelomic fluid the spirochætes invade practically all the organs of the tick, but are especially common in the salivary glands, Malpighian tubules and ovaries. Up to this point in their history there is a general agreement on the part of most investigators, but whereas Balfour and Hindle consider that the spirochætes segment into granules, which under varying conditions of environment and temperature may redevelop into spirochætes, Marchoux and Couvy, on the other hand, although admitting the disappearance of spirochætes from infected ticks, except possibly in the salivary ducts, are of the opinion that this disappearance is due to the spirochætes becoming attenuated owing to starvation. It is almost impossible to distinguish between the various types of granules found in cells of ticks, some of which are undoubtedly cell products, but in eggs laid by infected ticks and incubated at 37° C. the author (Hindle, 1912²) obtained intermediate stages between the short rounded form and the elongated spirochæte. The fact that infection could be produced in birds by the inoculation of eggs containing no obvious spirochætes is explained by Marchoux and Couvy (1913) on the supposition that they were too fine to be effectively stained, and by other critics on the supposition that they were present, but only in small numbers, and, in consequence, possibly overlooked. The author has carefully examined smears of eggs laid by infected *Argas*, using one-half of an egg-emulsion for the smear, and the other half for inoculation into chickens, and produced infection in birds that so far as could be determined were negative for spirochætes. The question is admittedly difficult to decide, for the chance of spirochætes being overlooked in such preparations is always present, but the author is of the opinion that the life-cycle indicated above is substantially correct.

Recently, Knowles, Das Gupta and Basu (1929), working in Calcutta, found that after ingestion by *Argas*, some spirochætes remained unchanged but others divided into short forms by multiple transverse fission. These short forms become very abundant and invade the coelomic fluid from the sixth day onwards and infect all the viscera. These so-called ' *tenue* ' forms are about one-third the length of the blood forms and are extremely thin ; they also show a fine terminal flagellum at each extremity about one-fourth to one-fifth the length of the spirochæte. Although present in all organs they accumulate in the salivary glands, where they develop into the normal blood forms, and infection is normally produced by the introduction of saliva containing spirochætes when the tick feeds. The coxal secretion, however, was also found to be infective but not so commonly as the salivary glands.

Other Spirochætes recorded from Birds.

Spirochæta lagopodis Fantham (1910) was recorded from the blood of the grouse, *Lagopus scoticus*. It is said to be 10 to 12 μ in length, rather

thick, with an undulating membrane and chromatic bands forming a spiral. Topfer, quoted by Mühlens and Hartmann (1906), found a spirochæte in a serum-agar culture of the blood of the little owl, *Athene noctua*, and Franchini (1924¹) recorded spirochætes from the blood of a snipe, *Gallinago gallinago*, in Italy.

BLOOD SPIROCHÆTES OCCURRING IN COLD-BLOODED ANIMALS.

Spirochætes have only rarely been recorded from the blood of snakes. Dobell (1910) described a species, *S. tropidonoti*, from the blood of *Tropidonotus stolatus* in Ceylon. It is said to resemble *S. duttoni*, and the dimensions are given as 15μ in length by 0.5μ in thickness. Neiva, da Cunha and Travassos (1914) described a short spirochæte occurring in the blood of *Tropidurus torquatus* in Brazil. It is 4 to 5μ in length with 3 to 5 spiral turns. The host feeds on arthropods, and the authors are of the opinion that the spirochæte may have an insect origin. Gleitsman and Mühlens have also recorded a spirochæte from the blood of a captive *Boa constrictor* at Hamburg.

In the case of amphibians, although spirochætes have been observed in the intestine, there seems to be no record of their having been found in the blood-stream.

Fishes are commonly infected with spirochætes in the intestinal tract, and various blood infections have also been recorded, some of which may be merely the result of gut contamination of the blood films. Dutton, Todd and Tobey (1906) described under the name *Spirochaeta jonesii*, a spirochæte found in the blood of a siluroid fish, *Clarias angolensis*, from the Gambia. The dimensions of this organism are 14 to 27μ in length by 0.6μ in breadth. The shape is peculiar, one end being pointed and the other folded backwards, suggesting some of the leptospiral forms. The spirals are large and very open. The periplast is distinct, and chromatic granules are present in the body of the parasite.

Duboscq and Lebailly (1912² and 1913) found in the blood of a marine fish, *Lepadogaster bimaculatus*, a spirochæte which is said to be quite distinct from the common intestinal forms. This organism, *S. perexilis*, closely resembles the intestinal spirochæte, *S. fallax*, but is more slender, less than 0.1μ in diameter. The size of the spirochæte varies from short forms 3μ in length, with only one and a half spirals, up to long forms 8μ in length with 6 spirals. *S. pelamidis* (Neumann, 1909¹) was found in the blood of *Pelamys sarda*. Its average length is 9 to 10μ , with 5 spirals. This species closely resembles the common intestinal species, *S. gadi*, but is slightly shorter and thicker. *Spirochaeta fallax* Duboscq and Lebailly, was recorded by Henry (1910) from the blood of a pollack, *Merlangus pollachius*, and subsequently described by Duboscq and Lebailly (1912¹). It is a small spirochæte ranging from short forms 4 to 5μ in length, with 3 spirals, up to longer forms 11μ in length, with 8 spirals. It is said to be common in the heart and veins, especially the rectal vein, but absent from the arterial blood.

Duboscq and Lebailly (1913) have made an extensive study of fish spirochætes and find that the intestinal forms penetrate the epithelial cells of the rectum and also enter the lymphatic spaces, and sometimes the blood-vessels (Fig. 29).

In addition to the above-mentioned examples, spirochætes have also been recorded from the blood of the following species of fish: *Gadus minutus* L., *Gadus biscus* L., *Onos mustela* L., *Blennius pholis* L. and *Lepadogaster gouani* Lacép. With the exception of *Clarias angolensis* all these species contain spirochætes in the rectum, and the question arises whether these blood forms are not derived from the intestine. It was found, however, that both true blood spirochætes and intestinal forms may be present, the former being much finer than the latter. They may also be distinguished by the length of time they survive in the blood. The intestinal forms very soon become non-motile and degenerate, whilst the blood spirochætes remain motile for at least 24 hours and in cover-glass preparations may live for 48 hours.

REFERENCES.

- ATKEY, O. P. H., 1929, *Bull. Off. int. Hyg. pub.*, **21**, 1932.
 BALFOUR, A., 1908 and 1911¹, *Third Report of Wellcome Research Labs.*, Khartoum, 38, and *Fourth Report*, Vol. A, p. 76; 1911², *J. Trop. Med.*, **14**, 113; 1914, *Trans. XVII Int. Cong. Med.*, London, Sect. 21, Part 2, p. 275.
 BATES, L. B., DUNN, L. H. & ST. JOHN, J. H., 1921, *Amer. J. Trop. Med.*, **1**, 183.
 BATES, L. B. & ST. JOHN, J. H., 1922, *J. Amer. Med. Ass.*, **79**, 575.
 BEKENSKY, P., 1916, *Rec. Méd. vét.*, **92**, 545.
 BOSSELUT, R., 1925, *Bull. Soc. Path. exotique*, **18**, 702.
 BREEZE, G. R., 1909, *J. Trop. Med.*, **12**, 98.
 BREINL, A. & KINGHORN, A., 1906, *Mem. Lpool. Sch. Trop. Med.*, **21**.
 BRIGGS, L. H., 1922, *J. Amer. Med. Ass.*, **79**, 941.
 BROWN, H. C., 1913, *Relapsing Fever United Provinces* (local publication), India.
 BROWSE, G. V., 1912, *Ind. Med. Gaz.*, **47**, 387.
 BRUCE, D., HAMERTON, A. E., BATEMAN, H. R., MACKIE, F. P. & LADY BRUCE, 1911, *Rep. Sleeping Sickness Commission*, Lond., **11**, 184.
 BRUMPT, E., 1908, *Bull. Soc. Path. exotique*, **1**, 577; 1909, *ibid.*, **2**, 285; 1919, *ibid.*, **12**, 748; 1926, *C.R. Acad. Sci.*, Paris, **183**, 1139; 1927, *Précis de Parasitologie*, 4th ed., Paris (Masson et Cie).
 BRUMPT, E. & FOLEY, 1908, *C.R. Soc. Biol.*, Paris, **65**, 132.
 BRUYNOGHE, R., 1928, *J. State Med.*, **36**, 3.
 DE BUEN, S., 1926, *Ann. Parasit.*, **4**, 185.
 BUSCHKE, A. & KROO, H., 1923, *Deuts. med. Wschr.*, **49**, 1435.
 BYKOWA, O., 1926, *Virchows Arch.*, **280**, 169.
 CAFFEY, P. J., 1926, *Trans. Soc. Trop. Med. Hyg.*, Lond., **20**, 195.
 CARPANO, M., 1913, *Ann. Igi. (sper.)*, **23**, 215; 1929, *Clin. vet. Milano*, **52**, 339.
 CARTER, H. V., 1877, *Med.-Chir. Trans.*, **60**, 265; 1882, *Spirillum Fever*, London, J. & A. Churchill; 1887, *Sci. Mem. Med. Sanit. Depts. Ind.*, **3**, 45.
 CARTER, R. M., 1907, *Ann. Trop. Med. Parasit.*, **1**, 157; 1908, *Ind. Med. Gaz.*, **43**, 370.
 CASTELLANI, A. & CHALMERS, A. J., 1910, *Manual of Tropical Medicine*, 1st Edit., London (Baillière, Tindall & Cox).
 CHAPCHEFF, C., 1925, *Bull. Soc. Path. exotique*, **18**, 97.
 COHN, F., 1875, *Beitr. Biol. Pfl.*, **1**, iii, 141.
 COLES, A. C., 1914, *Parasitology*, **7**, 17.
 COLLIER, W. A., 1926, *Die Methoden der Spirochätenforschung*, in Abderhalden's *Handbuch der biologischen Arbeitsmethoden*, Sect. 2, Part 3.
 COOK, A. R., 1904, *J. Trop. Med.*, **7**, 24.

- Cragg, F. W., 1922, *Trans. Soc. Trop. Med. Hyg.*, Lond., **15**, 236.
- Crawley, H., 1916, *J. Parasit.*, **2**, 87.
- Cropper, J., 1905, *Brit. Med. J.*, ii, 190.
- Cunningham, J., 1925, *Trans. Soc. Trop. Med. Hyg.*, Lond., **19**, 11.
- Delanoë, P., 1929, *Arch. Inst. Pasteur Tunis*, **18**, 133, 272.
- Deutz, 1912, *Hyg. Rdsch.*, **22**, 1017.
- Dobell, C. C., 1910, *Spolia zeylan.*, **7**, 65.
- Dodd, S., 1906, *J. Comp. Path.*, **19**, 318.
- Drake-Brockman, R. E., 1915, *Trans. Soc. Trop. Med. Hyg.*, Lond., **8**, 201.
- Dschunkowsky, E., 1913, *Deuts. med. Wschr.*, **39**, 419.
- Duboscq, O. & Lebailly, C., 1912¹, *C.R. Acad. Sci.*, Paris, **154**, 662; 1912², *ibid.*, **154**, 835; 1913, *Arch. Zool. exp. gén.*, **52**, 9.
- Dunn, L. H., 1923, *Amer. J. Trop. Med.*, **3**, 345; 1927, *J. Parasit.*, **13**, 177.
- Dutton, J. E. & Todd, J. L., 1905, *Mem. Lpool. Sch. Trop. Med.*, **17**, and *Brit. Med. J.*, ii, 1259; 1907, *Lancet*, Lond., ii, 1523.
- Dutton, J. E., Todd, J. L. & Tobey, E. N., 1906, *J. Med. Res.*, **15**, 491.
- Fantham, H. B., 1910, *Proc. Zool. Soc. Lond.*, 692.
- Fontana, A., 1912, *Derm. Wschr.*, **55**, 1003.
- Franchini, G., 1924¹, *Ann. Inst. Pasteur*, **38**, 470; 1924², *Bull. Soc. Path. exotique*, **17**, 884.
- Fülleborn, F. & Mayer, M., 1908, *Arch. Schiffs- u. Tropenhyg.*, **12**, 31.
- Gasperi, F. de, 1912, *Bull. Soc. Path. exotique*, **5**, 589.
- Gerlach, F., 1925, *Zbl. Bakt.*, Abt. I, Orig., **94**, 45.
- Gill, C. A., 1922, *Ind. J. Med. Res.*, **9**, 747.
- Gonder, R., 1907, *Arb. GesundhAmt.*, Berl., **27**, 406.
- Gózóny, L., 1911, *Zbl. Bakt.*, Abt. I, Orig., **57**, 535.
- Gray, J. D. A., 1928, *Ann. Trop. Med. Parasit.*, **22**, 273.
- Henry, H., 1910, *J. Path. Bact.*, **14**, 463; 1912, *ibid.*, **17**, 160.
- Heronimus, E. S., 1928, *Zbl. Bakt.*, Abt. I, Orig., **105**, 394.
- Hill, L., 1904, *J. Trop. Med.*, **7**, 35.
- Hindle, E., 1911, *Parasitology*, **4**, 133; 1912¹, *Proc. Camb. Phil. Soc.*, **16**, 457; 1912², *Parasitology*, **4**, 463.
- Hoffmann, W. H., 1921, *Trans. 4th Cong. Far-East. Ass. Trop. Med.*, Batavia, p. 42.
- James, S. P., 1905, *Sci. Mem. Med. Sanit. Depts. Ind.*, N.S. **13**, Calcutta.
- Johannessohn, F., 1926, *Med. Klinik*, **22**, 1614.
- Karlinski, J., 1902, *Zbl. Bakt.*, Abt. I, Orig., **31**, 566.
- Kerrest, J., Gambier, A. & Bouron, A., 1922, *Bull. Soc. Path. exotique*, **15**, 320.
- King, W. E., Baeslack, F. W. & Hoffmann, G. L., 1913, *J. Infect. Dis.*, **12**, 206.
- Kleine, F. K. & Eckard, B., 1913, *Z. Hyg. InfektKr.*, **74**, 389.
- Knowles, R., 1929, *Ann. Rep. Calcutta Sch. Trop. Med. for 1928*, p. 34.
- Knowles, R., Gupta, B. M. Das & Basu, B. C., 1929, *Trans. 7th Cong. Far-East. Ass. Trop. Med.*, British India, 1927, **2**, 573.
- Knuth, P. & du Toit, P. J., 1921, *Die Spirochätosen*, in Mense's *Tropenkrankheiten*, 2nd ed., **6**, 543.
- Koch, R., 1906, *Berl. klin. Wschr.*, **43**, 185.
- Kritschewski, J. L., 1927, *Klin. Wschr.*, **6**, 1370.
- Kritschewski, T. L. & Rubinstein, P. L., 1927, *Z. Immunforsch.*, **51**, 27.
- Lapidari, M. & Sparrow, H., 1928, *Arch. Inst. Pasteur Tunis*, **17**, 191.
- Laveran, A., 1903, *C.R. Acad. Sci.*, Paris, **136**, 939.
- Lebedjewa, M. N., 1929, *Z. Immunforsch.*, **61**, 505.
- Lebert, H., 1874, *Handb. Spec. Path. u. Therap.*, hrsg. H. v. Ziemssen, **2**, i, 243. Leipzig (F. C. W. Vogel).
- Leger, A., 1917, *Bull. Soc. Path. exotique*, **10**, 280.
- Leger, M., 1923, *Rev. Méd. Angola*, Special No. of 1^o Congr. Med. Trop. Africa Occ., **4**, 279.
- Leishman, W. B., 1910, *Trans. Soc. Trop. Med. Hyg.*, Lond., **3**, 77; 1920, *Lancet*, Lond., ii, 1237.
- Levaditi, C. & Anderson, T. E., 1928, *C.R. Acad. Sci.*, Paris, **186**, 653; 1929, *C.R. Soc. Biol.*, Paris, **100**, 1121.
- Levaditi, C. & Lange, F., 1905, *C.R. Soc. Biol.*, Paris, **58**, 843.
- Levaditi, C. & Manouélian, 1907, *Ann. Inst. Pasteur*, **21**, 295.

- LINGARD, A., 1907, *J. Trop. Vet. Sci.*, **2**, 261.
 MCCULLOCH, W. E., 1925, *J. Trop. Med.*, **28**, 332.
 MACFIE, J. W. S., 1914, *Ann. Trop. Med. Parasit.*, **8**, 439; 1916, *ibid.*, **10**, 305.
 MACFIE, J. W. S. & YORKE, W., 1917, *Ann. Trop. Med. Parasit.*, **11**, 81.
 MACKIE, F. P., 1907, *Brit. Med. J.*, ii, 1706; 1920, *ibid.*, i, 380.
 MANTEUFEL, 1908, *Arb. Gesundheitsamt.*, Berl., **29**, 355.
 MARCHOUX, E. & COUVY, L., 1913, *Ann. Inst. Pasteur*, **27**, 450 and 620.
 MARCHOUX, E. & SALIMBENI, A., 1903, *Ann. Inst. Pasteur*, **17**, 569.
 MASON, F. E., 1916, *Min. Agric. Vet. Serv., Ann. Rep.*, 1915, Cairo, 34.
 MATHIS, C., 1928, *Bull. Soc. Path. exotique*, **21**, 472 and 585.
 MATHIS, C. & LEGER, M., 1911¹, *Recherches de parasitologie et de pathologie humaines et animales au Tonkin*, Paris; 1911², *C.R. Soc. Biol.*, Paris, **70**, 212.
 MAYER, M., 1914, *Arch. Schiffs- u. Tropenhyg.*, **18**, 254.
 MELENEY, H. E., 1927, *Abh. Ausl. d. sk.*, **26**, 328.
 MORIN, A. & GENEVRAY, J., 1926, *Bull. Soc. Path. exotique*, **19**, 529.
 MOUZELS, P., 1913, *Ann. Hyg. Méd. col.*, **16**, 249.
 MÜHLENS, P., 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, 2nd ed., Jena, **7**, 864 and 921; 1930, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **7**, 383.
 MÜHLENS, P. & HARTMANN, M., 1906, *Z. Hyg. InfektKr.*, **55**, 81.
 NATTAN-LARRIER, L., 1909, *Bull. Soc. Path. exotique*, **2**, 239; 1911, *Ann. Inst. Pasteur*, **25**, 739.
 NEIVA, A., CUNHA, A. M. DA & TRAVASSOS, L., 1914, *Mem. Inst. Oswaldo Cruz*, **6**, 180.
 NEUMANN, R. O., 1909¹, *Z. Hyg. InfektKr.*, **64**, 1; 1909², *Münch. med. Wschr.*, **56**, 477.
 NEUMANN, R. O. & MAYER, M., 1914, *Atlas und Lehrbuch wichtiger tierischer Parasiten und ihrer Überträger*, Lehmann's Med. Atlanten, **11**, 276.
 NICHOLSON, F. D., 1919, *Brit. Med. J.*, ii, 811.
 NICOLLE, C., 1907, *C.R. Soc. Biol.*, Paris, **63**, 213.
 NICOLLE, C. & ANDERSON, C., 1926, *C.R. Acad. Sci.*, Paris, **182**, 1450; 1927, *Bull. Inst. Pasteur*, **25**, 657; 1928, *Arch. Inst. Pasteur Tunis*, **17**, 106; 1929, *ibid.*, **18**, 188.
 NICOLLE, C., ANDERSON, C. & COLAS-BELCOUR, J., 1927, *C.R. Acad. Sci.*, Paris, **185**, 334.
 NICOLLE, C., ANDERSON, C. & HORNUS, P., 1929, *Arch. Inst. Pasteur Tunis*, **18**, 163.
 NICOLLE, C., BLAIZOT, L. & CONSEIL, E., 1913, *Ann. Inst. Pasteur*, **27**, 204.
 NICOLLE, C. & COMTE, C., 1906, *Ann. Inst. Pasteur*, **20**, 311.
 NOGUCHI, H., 1912, *J. Exp. Med.*, **16**, 199 and 620.
 NOGUE, 1925, *Ann. Méd. Pharm. col.*, **23**, 445.
 NOVY, F. G., 1907, *Proc. Path. Soc. Philad.*, N.S. **10**, 1.
 NOVY, F. G. & KNAPP, R. E., 1906, *J. Infect. Dis.*, **3**, 291.
 NUTTALL, G. H. F., 1908¹, *Parasitology*, **1**, 143; 1908², *J. Inst. Publ. Hlth.*, Lond., **16**, 385.
 OBERMEIER, O., 1873, *Zbl. med. Wiss.*, **11**, 145.
 PARROT, L., 1920, *Bull. Soc. Path. exotique*, **13**, 647.
 PETTIT, A., 1928, *Contribution à l'étude des Spirochétidés*, Vanves.
 PICKOUL, I. N., 1928, *Russ. J. Trop. Med.*, **6**, 612.
 PLAUT & STEINER, 1919, *Arch. Psychiat. Nervenkr.*, **61**, 458.
 PLIMMER, H. G., 1912, *Proc. Zool. Soc. Lond.*, Part 2, 406.
 DEL PRADO, E., 1920, *An. Fac. Med. Lima*, **3**, 26 and 134.
 PRIGGE, R., 1926, *Deuts. med. Wschr.*, **52**, 356.
 PRIGGE, R. & ROTHERMUNDT, M., 1928, *Z. Hyg. InfektKr.*, **108**, 398.
 PROWAZEK, S. VON, 1907, *Arb. Gesundheitsamt.*, Berl., **26**, 23.
 RANKEN, H. S., 1912, *Brit. Med. J.*, i, 1482.
 ROSENHOLZ, H. P., 1927, *Zbl. Bakt.*, Abt. I, Orig., **102**, 179.
 ROSENHOLZ, H. P., OWSJANNIKOWA, O. W. & TREFILOW, I. A., 1928, *Russ. J. Trop. Med.*, **6**, 280.
 ROSS, P. H. & MILNE, A. D., 1904, *Brit. Med. J.*, ii, 1453.
 RUSSELL, A. J. H., AYYAR, A. & UBHAYA, 1924, *Ind. J. Med. Res.*, **11**, 791.
 SAGEL, W., 1928, *Arch. Schiffs- u. Tropenhyg.*, **32**, Beih. 6, 283.

- SAKHAROFF, N., 1891, *Ann. Inst. Pasteur*, **5**, 564.
 SANARELLI, G., 1927, *Ann. Inst. Pasteur*, **41**, 679.
 SANGIORGI, G., 1913, *Pathologica*, **5**, 428.
 SAVINI, E., 1923, *C.R. Soc. Biol.*, Paris, **88**, 956.
 SCHELLACK, C., 1907, *Arb. Gesundh.Amt.*, Berl., **27**, 364.
 SCHILLING, C., 1906, *Rückfallfieber*, in Mense's *Handb. d. Tropenkr.*, **3**, 668.
 SCHILLING, C. & RONDONI, P., 1913, *Z. ImmunForsch.*, Tl. I, Orig., **18**, 651.
 SCHLOSSBERGER, H. & WICHMANN, F. W., 1929, *Z. Hyg. InfektKr.*, **109**, 493.
 SCHREUS, H. T. & WEISBECKER, H., 1926, *Deuts. med. Wschr.*, **52**, 1902.
 SCHUBERG, A. & KUHN, P., 1911, *Arb. Gesundh.Amt.*, **31**, 377.
 SCHUBERG & MANTEUFEL, 1910, *Z. ImmunForsch.*, Tl. I, Orig., **4**, 512.
 SEGUIN, P., 1930, *C.R. Soc. Biol.*, Paris, **104**, 247.
 SELWYN-CLARKE, P. S., LE FANU, G. H. & INGRAM, A., 1923, *Ann. Trop. Med. Parasit.*, **17**, 389.
 SERGENT, ED. & FOLEY, H., 1908, *Bull. Soc. Path. exotique*, **1**, 174; 1910, *Ann. Inst. Pasteur*, **24**, 337.
 SMITH, C. H. & GRAHAM, G. F., 1913, *Ind. Med. Gaz.*, **48**, 381.
 SMITH, G. U., 1909, *On some cases of relapsing fever in Egypt and the question of carriage by domestic vermin*, London.
 STEEN, R. & TOWNSEND, R. S., 1913, *Ind. Med. Gaz.*, **48**, 338.
 STEPHENS, J. W. W. & CHRISTOPHERS, S. R., 1905, *The practical study of malaria and other blood parasites*, Liverpool (Williams and Norgate); 1908, *ibid.*, 3rd ed.
 ST. JOHN, J. H. & BATES, L. B., 1922, *Amer. J. Trop. Med.*, **2**, 251.
 THEILER, A., 1905, *Proc. Roy. Soc.*, B, **76**, 504; 1909, *Bull. Soc. Path. exotique*, **2**, 293.
 THIROUX, A. & DUFOUGERÉ, W., 1910, *Bull. Soc. Path. exotique*, **3**, 23.
 TODD, J., 1930, *Brit. Med. J.*, i, 312.
 TODD, J. L. & WOLBACH, S. B., 1912, *J. Med. Res.*, **26**, 195.
 TOMIOKA, Y., 1924, *Zbl. Bakt.*, Abt. I, Orig., **92**, 41.
 TOYODA, H., 1913, *Z. Hyg. InfektKr.*, **76**, 313; 1919, *Kitasato Arch.*, **3**, 42.
 VIANNA, G., DE FIGUEIREDO & CRUZ, B. G., 1912, *Brazil-med.*, **26**, 912.
 VINZENT, R., 1927, *Ann. Inst. Pasteur*, **41**, 131.
 WENYON, C. M., 1926, *Protozoology*, London (Bailliére, Tindall & Cox).
 WITTROCK, O., 1913, *Z. Hyg. InfektKr.*, **74**, 55.
 WRIGHT, H. D. & HAROLD, C. H. H., 1920, *R.A.M.C. Jl.*, **35**, 203.
 ZLATOGOROFF, 1923, *Deuts. med. Wschr.*, **49**, 973.

CHAPTER VII. SYPHILIS.

BY L. W. HARRISON (ST. THOMAS'S HOSPITAL, LONDON) ;
WITH SECTIONS BY W. BULLOCH (LONDON HOSPITAL) AND
EDWARD HINDLE, THE BEIT RESEARCH FELLOW IN TROPICAL
MEDICINE (WELLCOME BUREAU OF SCIENTIFIC RESEARCH).

History.

BY W. BULLOCH.

SYPHILIS is a typical instance of an infective disease, and it is not surprising that it was early made the subject of bacteriological study. At first the methods employed for its elucidation were incomplete and the results confusing. Nothing can be gained by referring to them now. For a short period considerable notoriety was gained by S. Lustgarten (1884, 1885), who, by a certain staining method demonstrated bacilli, particularly in the secretions of syphilitic sores of the genitals, but also in other sites. Lustgarten's 'Syphilis bacilli' were also seen by some other workers, but it was shown by Alvarez and Tavel (1885) that similar if not identical bacilli occurred in the normal secretions of the genital organs and their 'Smegma bacillus' took its place in bacteriological literature and was further investigated by H. Bitter (1886), Neufeld (1901) and exhaustively by A. Weber (1902). The ætiology of syphilis remained unknown and so unsuccessful were the bacteriologists in their efforts that for some years it appeared to be almost an insoluble problem. A new chapter was, however, begun by the studies of Metchnikoff and Roux, who, in a series of important memoirs (1903-6), showed conclusively that syphilis is communicable to certain monkeys. Their results were rapidly confirmed by Lassar (1903), A. Neisser (1904) and Finger and Landsteiner (1906). E. Bertarelli (1906, 1907) demonstrated the production of syphilis in the eye of the rabbit, and Parodi (1907) in the testicle of the same animal. It was rendered probable by Klingmüller and Baermann (1904) in filtration experiments that the virus of syphilis was particulate and the actual virus was found by F. Schaudinn and E. Hoffmann (1905¹) (April 10th) in the form of a spiral organism of characteristic appearance. The fundamental discovery of Schaudinn and Hoffmann was immediately confirmed all over the world and a great flood of literature was published on the subject. The recognition of the organism by dark-ground illumination was suggested by Landsteiner and Mucha (1906), and Levaditi (1905) developed an important technique for its demonstration in the tissues. The cultivation of the spirochæte of syphilis in a pure state was found to be very difficult, but was successfully attained by Noguchi (1911, 1912), who also discovered the organism in the

brains of general paralytics (Noguchi and Moore, 1913). The serological diagnosis of syphilis known as the Wassermann reaction was based on the important discovery of complement fixation which was made by Bordet and Gengou (1901). A. Wassermann, Neisser and Bruck (1906) applied the Bordet-Gengou principle to the diagnosis of syphilis in apes and Wassermann, Neisser, Bruck and Schucht (1906) to the sero-diagnosis of syphilis in man. From this time an unprecedented amount on the 'Wassermann reaction' has been published, but this cannot be further referred to here. The modern treatment of syphilis by chemotherapeutic agents such as arsenicals was almost wholly due to the efforts of Ehrlich, with Berthelm and Hata as collaborators, and culminated with his publications on 'Salvarsan' in 1910.

REFERENCES.

- ALVAREZ & TAVEL, 1885, *Arch. physiol. norm. path.*, 3 s., **6**, 303.
 BERTARELLI, E., 1906, *Zbl. Bakt.*, Abt. I, Orig., **41**, 320; 1907, *ibid.*, **43**, 167, 23
 BITTER, H., 1886, *Arch. path. Anat.*, **106**, 209.
 BORDET, J. & GENGOU, O., 1901, *Ann. Inst. Pasteur*, **15**, 289.
 EHRLICH, P. & HATA, S., 1910, *Die experimentelle Chemotherapie der Spirilloxen...*, Berlin (Springer).
 FINGER, E. & LANDSTEINER, K., 1906, *Arch. Derm. Syph.*, Wien, **78**, 335; **81**, 147.
 KLINGMÜLLER, V. & BAERMANN, G., 1904, *Deuts. med. Wschr.*, **30**, 766.
 LANDSTEINER, K. & MUCHA, V., 1906, *Wien. klin. Wschr.*, **19**, 1349.
 LASSAR, O., 1903, *Berl. klin. Wschr.*, **40**, 1189.
 LEVADITI, 1905, *C.R. Soc. Biol.*, Paris, **59**, 326.
 LUSTGARTEN, S., 1884, *Wien. med. Wschr.*, **34**, 1389; 1885, *Med. Jahrb.*, Wien, **15**, 89, 4 pl.
 METCHNIKOFF, E. & ROUX, E., 1903, *Ann. Inst. Pasteur*, **17**, 809; 1904, *ibid.*, **18**, 1, 657; 1905, *ibid.*, **19**, 673; 1906, *ibid.*, **20**, 785.
 NEISSER, A., 1904, *Deuts. med. Wschr.*, **30**, 1369, 1431.
 NEUFELD, L., 1901, *Arch. Hyg.*, Berl., **39**, 184.
 NOGUCHI, H., 1911, *J. Exp. Med.*, **14**, 99; 1912, *ibid.*, **15**, 90.
 NOGUCHI, H. & MOORE, J. W., 1913, *J. Exp. Med.*, **17**, 232.
 PARODI, U., 1907, *Zbl. Bakt.*, Abt. I, Orig., **44**, 428.
 SCHAUDINN, F. & HOFFMANN, E., 1905¹, *Arb. GesundhAmt.*, Berl., **22**, 527; 1905², *Deuts. med. Wschr.*, **31**, 711.
 WASSERMANN, A., NEISSER, A. & BRUCK, C., 1906, *Deuts. med. Wschr.*, **32**, 745.
 WASSERMANN, A., NEISSER, A., BRUCK, C. & SCHUCHT, A., 1906, *Z. Hyg. InfektKr.*, **55**, 451.
 WEBER, A., 1902, *Arb. GesundhAmt.*, Berl., **19**, 251.

W. B.

Nomenclature.

Schaudinn and Hoffmann first termed the organism *Spirochaeta pallida*. Later, Schaudinn (1905¹) accepted the suggestion of Vuillemin that it should be *Spirochaeta pallidum*. As, however, he inclined to the view that the organism was a protozoon, and it was found that the name *Spirochaeta* had in 1893 been given to another protozoon, he (1905²) invented the name *Treponema pallidum*. Dobell (1918), being of opinion that the organism belonged to the vegetable kingdom, repropounded the name *Spirochaeta*, but in 1926, having verified references supplied by Stiles to the effect that the name had been applied in 1836 to a vegetable genus of the Lauraceae,

he concluded that it should be called *Treponema pallidum*. Various other names have been suggested, but it is commonly known as either *Spirochaeta pallida* or *Treponema pallidum*, and as the term 'spirochaete' has frequently to be used in speaking of its relationship to other organisms, it seems most convenient here to refer to it as '*S. pallida*'.

REFERENCES.

- DOBELL, C., 1918, *Med. Res. Comm. Spec. Rep. Ser.*, No. 19; 1926, *Parasitology*, **18**, 368.
 SCHAUDINN, F., 1905¹, *Berl. klin. Wschr.*, **42**, 1665; 1905², *ibid.*, 1728.
 SCHAUDINN, F. & HOFFMANN, E., 1905¹, *Arb. Gesundh.Amt.*, Berl., **22**, 527; 1905², *Deuts. med. Wschr.*, **31**, 711; 1905³, *Berl. klin. Wschr.*, **42**, 673.
 VUILLEMIN, P., 1905, *C.R. Acad. Sci.*, Paris, **140**, 1567.

General Characters of *S. pallida* and its Relation to Other Organisms.

S. pallida shares with other members of the group termed spirochaetes the character of being a spiral organism able to move forwards or backwards and to bend to a curve or an angle. The group contains a number of species, many able to lead an independent existence and many parasitic, a number of the parasitic being pathogenic. Many spirochaetes are considerably larger than *S. pallida*, some being 100μ to $150\mu \times 1\mu$, but there are few which are finer. A close relationship between *S. pallida* and other pathogenic spirochaetes seems to be indicated by the fact that, like these, it is more susceptible to the action of the arsenobenzene group of drugs administered to the host than are other organisms. Schaudinn regarded it as being related to trypanosomes, and these organisms are also susceptible to the action of arsenobenzene preparations, though less so than the pathogenic spirochaetes. On the other hand, trypanosomes are more susceptible to other remedies, e.g. tryparsamide, than is *S. pallida*. Two other spirochaetes, *S. pertenuis*, first seen in yaws lesions by Castellani (1905), and *S. cuniculi*, discovered by E. H. Ross (1912), must be closely related to *S. pallida* from which they are morphologically indistinguishable.

S. pertenuis is dealt with more particularly in Chapter X. *S. cuniculi* (see p. 283) was first seen by Ross in a disease of rabbits characterized by superficial sores on the genitals, anus and mouth. Its morphological characteristics were described by Bayon (1913), and it was again noticed by Arzt and Kerl (1914) in lesions affecting the genitals of many rabbits, but it was not named until 1920 when Jacobsthal proposed for it the name of *Spirochaeta paraluis-cuniculi*, which was contracted by Levaditi, Marie and Nicolau (1921) to *S. cuniculi*.

The interrelationship of *S. pallida*, *S. pertenuis* and *S. cuniculi* is not definitely known. They are susceptible in different degrees to the same remedies, and they differ chiefly in their effects. *S. pallida* is the most invasive of the deeper tissues, then *S. pertenuis* and lastly *S. cuniculi*, which, moreover, is non-infective for man or monkeys and produces far more superficial lesions in rabbits.

~ **Morphology.**

In the secretion from syphilitic lesions examined fresh under dark-ground illumination, *S. pallida* appears as a dead-white, delicate corkscrew-shaped organism, with clear-cut, very regular coils which are rather more opened out towards the ends, where the body of the organism is thinner. The ends appear to be pointed, rather like the end of a corkscrew. They are visible under ordinary conditions, and beyond them the spirochæte is prolonged by a very fine thread first described by Schaudinn (1907). He also saw in some specimens a splitting of these terminal threads, which he believed to be indicative of longitudinal fission, but the appearance might have been due to two spirochætes having become very closely entangled. In fresh specimens examined under ordinary conditions, when the fine terminal threads cannot be seen, their presence may be inferred from the movements of extraneous particles in tune with those of the spirochæte. The length of *S. pallida* varies from 4 to 14μ , or more, the thickness has been estimated at 0.25μ ; the distance from crest to crest of two coils at 1 to 1.2μ ; and the depth of the coils at 1 to 1.5μ , except towards the ends where they appear rather shallower.

MOTILITY.

S. pallida travels with either end foremost at a comparatively slow rate, so that it is easy to keep the same specimen in the field for hours together. On the other hand, especially when fresh caught, it can actively bend into a circle, to form a loop of its terminal coils, or to various angles, even a V. Its coils expand and contract, and it is generally agreed that it rotates round its long axis, though Jeantet and Kermorgant (1925), maintain that *S. pallida* and *S. cuniculi* do not rotate but progress by wave-like movements in one plane. These observations have not been confirmed.

STAINING.

The characteristic shape of *S. pallida* can be demonstrated by methods which stain the background, leaving the organism colourless. Many agents are used for the purpose, such as Indian ink, collargol and Congo-red, with which the secretion is mixed, spread on a slide and allowed to dry.

In films of secretion from syphilitic lesions *S. pallida* takes up aniline dyes only very slowly unless a mordant has been applied to the film, or gentle heat is used during the staining. The first method of staining employed was a modification of Giemsa's, and the staining was prolonged for 16 to 24 hours. This method was replaced by one in which, after preliminary fixation with alcohol, the dried film was covered with a 1 in 2 dilution of Giemsa's solution for an hour. Under these and the Leishman stain, *S. pallida* is usually dyed rose-pink. Preliminary fixation of the specimen with osmic acid vapour before it is spread has the advantage that the spirochætes are less distorted by the spreading of the film. Recently Bergel (1928¹) has called attention to the scarcity of *S. pallida* in specimens

which are 'fixed' with alcohol before staining compared with the numbers seen in the same secretion under dark-ground illumination, due, he believes, to solution of the lipoid content of many of the spirochætes by the alcohol.

Films of syphilitic secretion are readily stained by a silver method, a very satisfactory one being Tribondeau's modification of Fontana's, or Fontana's special method for staining the terminal threads. By any silver method the organism is blackened and slightly thickened. It was established by MacWeeney (1905), Lipschutz (1905) and others, and confirmed by Wilkes-Weiss (1924), that *S. pallida* is Gram-negative.

Stained specimens of *S. pallida* of syphilitic secretion show various modifications of the shape seen under dark-ground illumination, probably mainly due to distortion in spreading and drying the film. The coils are commonly more pulled out, especially in the middle. Sometimes exceptionally long spirochætes are seen with their middle portions drawn out and very thin, indicating probably a transverse division. Naturally, since the spirochæte can bend to almost any degree, specimens are seen in which the body has been fixed in such bent conditions. Sometimes short spirochætes are found with ends bent into circles giving a bulbous appearance. Entanglements of large numbers of spirochætes are seen more commonly in stained specimens than in fresh. By the silver method and also by Löffler's stain for flagella the fine end-threads mentioned above can be demonstrated. They are believed to be prolongations of the perioplast.

In sections of syphilitic tissue, which are commonly stained by a modification of Levaditi's silver method, the regularity of the coils is preserved better than in spread films, but they are rather thicker.

INVOLUTION FORMS.

Involution forms such as short, deformed fragments, swollen, coiled-up and clumped spirochætes, ring and star forms, have been described by various authors, but probably most of these atypical appearances are artefacts, entanglements of spirochætes, or spirochætes fixed when bent into various curves or angles.

The breaking up of *S. pallida* into granules, or the shedding of these by the organism has been described by some workers. Balfour (1911) observed shedding of granules by *S. pallida* in the secretion from a syphilitic lesion after the patient had been injected with '606' and suggested that they were resistant forms. Shortly afterwards, O'Farrell and Balfour (1911) observed granule shedding by *S. pallida* and by associated *S. refringens* in the secretion from syphilitic lesions examined under dark-ground illumination, particularly after an injection of '606'. Highly refractile granules were racing up and down the spirochætes and every now and again a granule was discharged, sometimes for a considerable distance. As a result of the granule-shedding certain of the spirochætes became faint, lost their silvery appearance and eventually came to rest, either as empty perioplasts, or as flattened cell membranes

containing one or two bright but motionless granules. A granule was sometimes seen at one or other extremity as though the expiring spirochæte had just failed to expel it. The authors thought the granules were of the nature of resistant spores.

Bergel (1928²) has described in syphilomata of the scrotum of rabbits a process of degeneration which he believed to be due to the action of the surrounding lymphocytes on the lipid content of the spirochætes.

Levaditi, Schoen and Sanchis-Bayarri (1927) have suggested that *S. pallida* has a life-cycle at one end of which is a minute granule of almost ultramicroscopic dimensions and at the other the fully formed spirochæte, while between are involution forms of very diverse shapes. They pointed first to the very high infectivity of glands of rabbits suffering from experimental syphilis and yet the rarity with which *S. pallida* is seen in the glands. This observation was made first by Truffi (1913), and at a later date Pearce and Brown (1922) found *S. pallida* microscopically only three times in 29 virulent glands of syphilitic rabbits. Levaditi and colleagues examined 33 glands of syphilitic rabbits, most of which were infective, and found no typical spirochæte, but only some doubtful forms in two or three specimens.

They made a histological study of grafts of these virulent glands, stained by the methods of Stempel and Armuzzi (1926) and of Levaditi, at varying intervals after implantation into the scrota of other rabbits, and described the changes which occurred in the grafts in two stages, pre-spirochætal and spirochætal. In the pre-spirochætal stage, which lasted approximately 45 days, nothing resembling *S. pallida* was seen but only silver-stained granules, for the most part within the fixed cells. This contrasted with the findings when a tissue already containing *S. pallida*, such as scrotal syphiloma or cornea affected with syphilitic keratitis, is grafted into the scrotum, since in the latter case *S. pallida* is seen constantly and multiplies steadily. Its absence from glandular grafts for many days after implantation is not, therefore, explained by the temporary disappearance of any spirochætes originally present. In the spirochætal stage the graft lost its glandular character, and was replaced by the elements of a syphiloma, viz. new-formed vessels, fibroblasts, fibrils, lymphocytes, plasma cells, and large numbers of giant cells, some of these containing as many as 40 nuclei. Around the larger giant cells were numerous typical *S. pallida*. Within them were typical spirochætes and also what the authors considered to be involution forms. Within the smaller giant cells and the fibroblasts only involution forms were seen. These involution forms were of various shapes and sizes and were considered by Levaditi to be stages in a life-cycle of *S. pallida*.

Krzyształowicz and Siedlecki (1905) described thick and thin spirals which they believed to be female and male forms respectively. Conjugation between the two types of spirals, and all stages of development from spirochætes to trypanosome-like forms were seen.

E. H. Ross and J. E. R. McDonagh respectively have described appearances which they considered to be stages in the life-cycle of *S. pallida*.

Meirowski (1925) has described peculiar buds on *S. pallida*, sometimes at one end, sometimes attached by stems to the body of the organism and sometimes free. His specimens were fixed with osmic acid vapour and stained with Pappenheim. He considered that these buds, which he had also seen in cultivated spirochætes, contained the nuclear substance of the organism and gave rise to fresh spirochætes. Grütz (1924) made similar observations in the case of cultures of *S. pallida* in half-inspissated human serum. Like Meirowski, he thought that *S. pallida* multiplies by budding and by transverse fission. There is no proof, however, that these presumed involution forms and stages in a life-cycle were not artefacts or merely fragments of tissue.

Variations from the typical form occur more commonly in cultures than in the tissues and seem to depend on the nature of the medium, such as thick (*refringens*-like) and thin forms, and spirochætes are sometimes seen which are thick for a portion of their length and thin for the rest of it. Again, however, the cultures may not have been pure.

REFERENCES.

- ARZT, L. & KERL, W., 1914, *Wien. klin. Wschr.*, **27**, 1053.
BALFOUR, A., 1911, *R.A.M.C. Jl.*, **16**, 695; 1912, *Brit. Med. J.*, i, 362.
BAYON, H., 1913, *Brit. Med. J.*, ii, 1159.
BERGEL, S., 1928¹, *Klin. Wschr.*, **7**, 1509; 1928², *ibid.*, **7**, 1681.
CASTELLANI, A., 1905, *Brit. Med. J.*, ii, 1330.
GRÜTZ, O., 1924, *Arch. Derm. Syph.*, Wien, **147**, 337.
JACOBSTHAL, E., 1920, *Derm. Wschr.*, **71**, 569.
JEANTET, P. & KERMORGANT, Y., 1925, *C.R. Soc. Biol.*, Paris, **92**, 1036.
KRZYSZTAŁOWICZ, F. & SIEDLECKI, M., 1905, *Bull. Acad. Sci. Cracov* (Nov.), **713**, cited from LEVADITI & ROCHÉ, 1909, *La Syphilis*, Paris, Masson, p. 172.
LEVADITI, C., MARIE, A. & NICOLAU, S., 1921, *C.R. Acad. Sci.*, Paris, **172**, 1542.
LEVADITI, C., SCHOEN, R. & SANCHIS-BAYARRI, V., 1927, *Bull. Acad. Méd.*, Paris, **98**, 149; 1928, *Ann. Inst. Pasteur*, **42**, 475.
LIPSCHÜTZ, B., 1905, *Deuts. med. Wschr.*, **31**, 1832.
MACWEENEY, E. J., 1905, *Brit. Med. J.*, i, 1262.
MEIROWSKI, 1925, *Arch. Derm. Syph.*, Wien, **149**, 1.
O'FARRELL, W. R. & BALFOUR, A., 1911, *R.A.M.C. Jl.*, **17**, 225.
PEARCE, L. & BROWN, W. H., 1922, *J. Exp. Med.*, **35**, 39.
ROSS, E. H., 1912, *Brit. Med. J.*, ii, 1651 (footnote).
SCHAUDINN, F., 1907, *Arb. Gesundh.Amt.*, Berl., **26**, 15 (posthumous publication).
STREMPER, R. & ARMUZZI, G., 1926, *Derm. Z.*, **48**, 267.
TRIBONDEAU, L., 1912, *Bull. Soc. franc. Derm. Syph.*, **23**, 474; 1917, *Ann. Inst. Pasteur*, **31**, 412.
TRUFFI, M., 1913, *Pathologica*, p. 316, cited from LEVADITI, SCHOEN & SANCHIS, BAYARRI.
WILKES-WEISS, D., 1923-4, *J. Lab. Clin. Med.*, **9**, 716.

Cultivation.

Schereschewsky (1909) appears to have been the first to claim success in the culture of *S. pallida*. He used semi-inspissated horse-serum into which he pushed pieces of tissue from syphilitic lesions. Later (1921),

by a modification of a method recommended by Sowade (see below), he was able to purify his cultures, and he also claimed some success in provoking syphilomata in rabbits by inoculation with his culture. Subsequently, Mühlens (1909), W. H. Hoffmann (1911^{1&3}), Noguchi (1911^{1&2}), Sowade (1912), Nakano (1912) and others, published methods of obtaining the organism in pure culture.

Most workers have cultivated from pieces of human syphilomata, employing chiefly primary chancres, papules and glands. Schereschewsky obtained a culture from the spleen of a syphilitic infant, and a few have been successful with secretion from syphilitic lesions. Noguchi's first successes, after many failures, were with pieces of syphilomata from rabbits.

PRIMARY CULTURE.

Cultivation is by no means easy, and failures have been far more numerous than successes. Since 1920 a large number of methods have been recommended. They agree in the principle, that, as Noguchi first pointed out, strictly anaerobic conditions are necessary, and for first cultures an inspissated medium containing serum (horse-, sheep-, calf-, pig- or rabbit-serum serves well), ascitic or hydrocele fluid has been mostly employed, but Noguchi used one part ascitic fluid with two of 2 per cent. slightly alkaline agar. The use of a solid medium enables the spirochæte to be isolated more easily from the primary mixed culture, as *S. pallida* has the faculty of outstripping these in penetration into the medium so that it can be picked out of the outlying parts of the growth.

Anaerobic conditions were supposed to be secured in most of the older methods of primary culture by symbiosis with other organisms—not a very satisfactory method according to modern views. Shmamine (1912) planted a piece of syphilitic tissue 5 cm. deep in a solid medium, usually covering with liquid paraffin, and incubating for 5 to 21 days at 37° C. before subculturing. The medium was horse-serum with an addition of 0.5 to 0.75 per cent. of sodium nucleinate. Through this CO₂ was bubbled for two or three minutes to make it clearer; the mixture was then heated to 60° C. for one hour on each of three days, and was finally inspissated at 70° C. until somewhat hard. Krantz (1924) pointed out that in an impure culture, some of the contaminating organisms favour, others inhibit, the growth of *S. pallida*, and that in a fluid culture medium the toxins of the unfavourable organisms tend to kill off the spirochæte. He therefore employed an inspissated serum medium with methylene blue as an indicator of the anaerobic condition, and found that after incubation for 8 to 14 days of a mixed culture, *S. pallida* was found at the junction of the blue with the blanched portion accompanied by favouring organisms. Reiter (1926) claimed good results with a medium consisting of rabbit-serum and saline with pieces of rabbit-brain. Noguchi, one of the chief claimants of successful cultivation of *S. pallida*, placed a piece of fresh sterile rabbit-kidney or testicle at the bottom of the medium and employed hydrogen and pyrogallol for anaerobiosis.

ISOLATION IN PURE CULTURE.

For isolation in pure culture many methods have been recommended. Noguchi (1911²) at first, and also Nakano (1912), made use of the ability of *S. pallida* to grow through a Berkefeld filter candle more quickly than the contaminating organisms, and W. H. Hoffmann (1911¹), Tomaszewski (1912) and others have taken advantage of the faculty of *S. pallida* to grow into the medium more quickly than other organisms. Bronfenbrenner (1914) used a 1/5,000 to 1/10,000 solution of salvarsan, and Sowade (1912) spirit, to kill off the contaminating organisms in a mixed culture.

Krantz (1924) first cultures in a thin serum—or ascitic or hydrocele fluid—agar with a reaction of pH 7·3 to 7·5 and with methylene blue indicator (see above). From a layer of growth of *S. pallida* and favouring organisms subcultures are then made into a fluid medium of reaction pH 7·3 to 7·5. After a few days' incubation, *S. pallida* is mostly at the bottom of the tube and a skin of the favouring bacteria at the top. A second subculture is now made from the bottom of the first one into another tube of the fluid medium, which is then covered with a layer of melted vaseline. The anaerobiosis now inhibits the growth of the favouring bacteria, but encourages that of *S. pallida*. For the fluid medium several mixtures succeeded, such as serum with pieces of boiled egg-white, ascitic fluid with 5 per cent sodium formate, ascitic fluid with boiled blood and water, and others.

Noguchi (1912), after many failures, devised a method by which *S. pallida*, after becoming accustomed to grow anaerobically in the more favourable solid medium, could migrate into a fluid medium without the anaerobic conditions being disturbed. A double tube was made up consisting of (a) a test-tube into the bottom of which was fused a piece of glass tubing, and (b) a larger test-tube, fitted with a perforated rubber bung, through which was passed the glass tube projecting from the bottom of the first test-tube (a), which was plugged with cotton-wool. After sterilization, the upper tube and bung were removed, two pieces of fresh sterile rabbit's kidney were put into the lower tube, following which the upper tube and bung were refitted as tightly as possible. The lower tube (b) was then filled with ascitic fluid, or a mixture of this and broth, by means of a capillary pipette which was passed through the upper tube and connecting tube into the lower tube. Two pieces of sterile tissue were then put into the upper tube, and the inoculation was performed by aspirating from a well-growing culture with a capillary pipette attached to pressure tubing and a syringe and ejecting partly into the fluid in the lower tube and partly around the tissue in the upper tube. After this the upper tube was filled with ascitic fluid agar, the ascitic fluid having been added to melted agar at 42° C. Some sterile paraffin was poured on the top of the agar to a depth of 3 cm., and incubation commenced at 37° C. Usually twelve double tubes were prepared at once. Following the suggestions conveyed by Krantz's work, one would now correct the pH of the two media employed in this method to 7·3 to 7·5 and pour a layer of melted

vaseline on the top of the agar. Although incubation is usually at 37° C., growth is said to proceed at room temperature.

CULTURAL CHARACTERISTICS.

In stab cultures a chain of colonies appears which looks more or less like a string of pearls. In shake cultures the medium either becomes turbid, or fine whitish points appear in it which enlarge gradually to cloudy balls. If the growth is in serum, this is not liquefied, and the growth is odourless. Subculture is carried out about every 10 to 14 days, but Noguchi stated that growth usually continues for about two months, and Krantz has subcultured after this time. The culture can survive a few transfers to plain agar. In liquid media growth is much slower, even when particular care is taken to secure anaerobiosis.

Considerable doubt of the identity of the spirochætes cultivated by the above workers and others with the organism of syphilis has recently been expressed in a review of the subject by Kast and Kolmer (1929). They state that they have entirely failed to cultivate *S. pallida* from lesions of rabbits and men by sixteen of the published methods, though they have often succeeded in growing saprophytic spirochætes from smegma and saliva. They consider 'that many of the alleged successful cultivations of *S. pallida* may have been growths of *pallida*-like saprophytic spirochætes'. They think that the cultures obtained by Noguchi, especially those from syphilitic rabbits, were more likely than most of the others to have been *S. pallida*, but remark that the majority of other investigators, including themselves, have not been able to obtain cultures by his technique. They had applied to other workers for their cultures, but, except for a Noguchi culture, had received none. Neither the Noguchi culture nor one of *S. microdentium* isolated by Kast would grow in Reiter's medium, and they thought that Reiter's cultures could not be identical with the Noguchi culture they had used. Kolmer and Kast must suppose that such tissues as spleen of syphilitic foetus, with which Schereschewsky claimed success, and cornea of syphilitic rabbit, used by Kroó and Schulze (1928), can be contaminated with *pallida*-like saprophytic spirochætes. Also that the *pallida*-like organisms can be pathogenic for rabbits.

As regards the pathogenicity of cultures, Bruckner and GalasESCO (1910) claimed to have produced syphilitic orchitis in rabbits and Sowade (1911) generalized syphilis by intravenous inoculation into rabbits, in both instances with mixed cultures. Noguchi (1911²) reported that two strains isolated in pure culture had been inoculated into rabbits' testicles, and the lesions produced had been identical histologically with those following inoculation with syphilitic material from human beings. Kroó, Schulze and Zander (1929) mention that inoculation of two rabbits with the eightieth subculture of their *S. pallida*, fifteen months after isolation, produced closed chancres in which numerous spirochætes were found. Mulzer and Nothhaas (1928) reported that they had cultivated by Reiter's

method a strain of *S. pallida* which retained its virulence for rabbits after 200 subcultures, and then after three passages through rabbits behaved like a biologically old strain, for it caused superinfection in rabbits primarily inoculated with strains which had had few animal passages. (Superinfection in rabbits is most easily effected when a heterologous strain is used which has been passaged through rabbits more times than that used for the primary inoculation.) In their review Kast and Kolmer quote successful inoculations of animals claimed by seven groups of workers, not including the two just mentioned, and failures by seven others.

The evidence for successful cultivation based upon the pathogenicity of the cultures certainly seems to be strong. On the other hand, as will be shown later, except in the case of rabbits, inoculations with killed cultures of *S. pallida* do not result in the serological response which follows true infection, though syphilitic serum has some agglutinating effect on cultural *S. pallida* and also some complement-fixing power with antigen made from it.

REFERENCES.

- BRONFENBRENNER, J., 1914, *Proc. Soc. Exp. Biol.*, N.Y., **12**, 136.
 BRUCKNER, I. & GALASESCO, P., 1910, *C.R. Soc. Biol.*, Paris, **68**, 684.
 HOFFMANN, W. H., 1911¹, *Z. Hyg. InfektKr.*, **68**, 27; 1911², *Deuts. med. Wschr.*, **37**, 1546; 1911³, *Berl. klin. Wschr.*, **48**, 2160.
 KAST, C. C. & KOLMER, J. A., 1929, *Amer. J. Syph.*, **13**, 419.
 KRANTZ, W., 1924, *Zbl. Bakt.*, Abt. I, Orig., **92**, 216.
 KROÓ, H. & SCHULZE, F. O., 1928, *Klin. Wschr.*, **7**, 246.
 KROÓ, H., SCHULZE, F. O. & ZANDER, I., 1929, *Klin. Wschr.*, **8**, 783.
 MÜHLENS, P., 1909, *Deuts. med. Wschr.*, **35**, 1261; 1910, *Klin. Jahrb.*, **23**, 339.
 MULZER, P. & NOTHHAAS, R., 1928, *Münch. med. Wschr.*, **75**, 169.
 NAKANO, H., 1912, *Deuts. med. Wschr.*, **38**, 1333.
 NOGUCHI, H., 1911¹, *J. Exp. Med.*, **14**, 99; 1911², *J. Amer. Med. Ass.*, **57**, 102; 1912, *J. Exp. Med.*, **15**, 201; 1917, *Amer. J. Syph.*, **1**, 261.
 REITER, H., 1926, *Klin. Wschr.*, **5**, 444.
 SCHERESCHESKY, J., 1909, *Deuts. med. Wschr.*, **35**, 835, 1260, 1652; 1912, *ibid.*, **38**, 1335.
 SHAMAMINE, T., 1912, *Zbl. Bakt.*, Abt. I, Orig., **65**, 311.
 SOWADE, H., 1911, *Deuts. med. Wschr.*, **37**, 1934; 1912, *ibid.*, **38**, 797.
 TOMASZEWSKI, E., 1912, *Berl. klin. Wschr.*, **49**, 1556.

Distribution and Persistence.

VIABILITY.

Beer (1906) first reported that between a slide and cover-slip he had seen *S. pallida* still motile after 33 days. In a wet specimen luted with vaseline which was prepared by Temp.-Capt. D. A. Stewart, R.A.M.C., in 1918, I saw *S. pallida* moving on the thirty-fifth day, and was later informed that the movement did not cease until the forty-fourth day. Lacy and Haythorn (1921) found the organisms still motile in autopsy material which had been kept in the refrigerator for 48 hours; they remained motile in a saline suspension of syphilitic rabbit's testicle juice sealed in tubes for 58 days, both at room temperature and in the refrigerator. Syphilitic autopsy material was infective 26 hours after death, chancre

24 hours after excision and syphilitic rabbit's testicle 24 hours, but not 54 hours, after excision. Hoffmann (1929) has collected records of several cases which show that *S. pallida* can retain its virulence in corpses sufficiently long to infect those performing autopsies. Considering, however, the number of autopsies on syphilitics and the small number of accidental infections during post-mortem examinations (20 certain and 18 probable collected by Hoffmann), it may be concluded that the virulence dies out in a few days. Zurhelle and Schleyer, quoted by Hoffmann, found that inoculations of 48 rabbits with material from dead syphilitic rabbits resulted in only 17 infections. Grigoriev and Jarisheva (1928) infected rabbits with liver and placenta of a syphilitic foetus removed 48 hours previously and kept in the refrigerator. Inoculations at 72 and 96 hours failed to infect. Noguchi (1917) reported that excised syphilitic tissues may remain infective even as long as 72 hours.

Resistance to cold and heat. Kissmeyer (1923) reported having subcultured *S. pallida* after keeping it at -16°C . for 3 months, but at 2°C . they died quickly. Cultural *S. pallida* were found by Akatsu to be killed in 5 minutes at 60°C . and 15 minutes at 55°C ., but Bronfenbrenner and Noguchi (1912-13) found that the lethal temperature was lower, viz. 45°C . for 7 to 10 minutes.

Resistance to chemicals. The following chemicals killed cultural *S. pallida*: Saponin, 1/75,000; sodium taurocholate and sodium glycocholate, 1/2,500; sodium cholate, 1/5,000; sodium oleate, 1/70,000; cobra lecithid, 1/1,000; phenol, 1/1,000 in 15 minutes; formalin, 1/500 in 15 minutes; and potassium permanganate, 1/5,000 in 15 minutes. Wilkes-Weiss and Weiss (1926), in attempts to purify cultures of *S. pallida* by chemical agents, found that treatment with selenium oxychloride 1/100 for 1 minute, trichloroacetic acid 1/100 for 15 minutes, and formaldehyde 1/20 for 5 minutes would kill *Staphylococcus aureus* and *B. coli* but not *S. pallida*.

Drying. It is agreed that *S. pallida* is quickly killed by drying.

DISTRIBUTION IN TISSUES OF ANIMALS.

The experiments of Brown and Pearce (1921^{1&2}) and numerous other workers have shown that, in rabbits, after a stage of generalized lesions, or without these, the virus is found most constantly in the lymph glands, where it remains for an indefinite period, probably the life of the animal. Uhlenhuth and Grossmann (1927) and others have shown, however, that sometimes when inoculation with lymph-gland material into other animals fails, inoculation with bone marrow or spleen may be successful. They recommend, therefore, that, in testing rabbits for persistence, or otherwise, of infection, e.g. after therapeutic experiments, an emulsion should be prepared not only from lymph glands but from organs and from bone-marrow. In mice the experiments of Schlossberger (1929) show that after inoculation the virus is distributed throughout the animals tissues, including brain, and remains so for many months.

DISTRIBUTION IN MAN AND PERSISTENCE OF INFECTIVITY.

In man, as is well known, the virus is distributed throughout the body and probably remains so as long as the infection persists, although, as will be shown, certain tissues tend more than others to react and show signs of disease. It seems probable that, after the stage of generalized lesions, *S. pallida* does not circulate freely in the blood-stream, and this may partly explain the fact that syphilitic persons become less and less contagious by sexual intercourse after the first year until by about the fifth year they are usually, though not always, safe in this respect. The infectivity of women for their offspring notoriously persists far longer than that for their consorts, and within the child-bearing period, there is probably no limit to the length of time a woman may convey syphilis to the foetus *in utero*. An example of this is the fact that a congenital syphilitic may give birth to a syphilitic infant. At the same time the tendency to transmit the disease does diminish, as is expressed in the commonly stated sequence of miscarriages, still-births, infants born alive but dying in infancy and infants who show no sign, but in later years as children, adolescents or adults present clinical evidence of disease. As is well known this sequence is by no means invariable, since apparently healthy infants may be born between syphilitic miscarriages and still-births, and of twins, as my own experience and that of others shows, one may be healthy but the other syphilitic.

REFERENCES.

- BEER, A., 1906, *Deuts. med. Wschr.*, **32**, 1192.
 BRONFENBRENNER, B. J. & NOGUCHI, H., 1912-13, *J. Pharmacol.*, **4**, 333.
 BROWN, W. H. & PEARCE, L., 1921¹, *J. Exp. Med.*, **34**, 185; 1921², *Amer. J. Syph.*, **5**, 1.
 GRIGORIEV, P. S. & JARISHEVA, K. G., 1928, *Klin. J. Saratov Univ.*, **5**, 83, cited from *J. Amer. Med. Ass.*, 1929, **92**, 96.
 HOFFMANN, E., 1929, *Derm. Wschr.*, **88**, 284.
 KISSMEYER, A., 1923, *Münch. med. Wschr.*, **70**, 775.
 LACY, G. R. & HAYTHORN, S. R., 1921, *Amer. J. Syph.*, **5**, 401.
 NOGUCHI, H., 1917, *Amer. J. Syph.*, **1**, 261.
 SCHLOSSBERGER, H., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 237*; 1929, *Med. Klinik.*, **25**, 307.
 UHLENHUTH, P. & GROSSMANN, H., 1927, *Klin. Wschr.*, **6**, 292.
 WILKES-WEISS, D. & WEISS, C., 1926, *J. Infect. Dis.*, **38**, 281.

Pathogenic Action.

METHODS OF TRANSFERENCE.

From what has been written, it will easily be understood that, to be successful, transfer of infection from one host to another must be direct, or almost so. In the case of man instances have been recorded of transfer through the medium of external objects such as a blow-pipe in glass-blowing, a drinking-glass, or a tobacco-pipe, but here the virus had been deposited on the article only a minute or so beforehand.

In man the organism gains entrance through two main routes: (a) through any breach in the skin or the mucous membranes of the

mouth, nasopharynx, rectum, vagina or urethra ; (b) directly into the blood-stream, as in congenital syphilis and in accidental infection by transfusion. There is no evidence in man that the organism can penetrate the intact skin or mucous membrane, but in rabbits Reasoner (1917) and Brown and Pearce (1924) demonstrated invasion through the mucous membrane of the prepuce and the conjunctiva, which, as far as they could judge, were intact. Invasion through the œsophagus, stomach and intestines above the lower third of the rectum has never been proved.

The channel by which infection is transferred to the foetus *in utero* is not known with certainty. In a recent article, Matzenauer (1929) has recalled that twenty-five years ago when he first advanced the theory that infection of the foetus is always maternal, he received little support, but events have proved it to be correct. What have been claimed to be exceptions to Colle's law that the mother of a congenital syphilitic infant does not acquire syphilis from it have been published, but have not withstood critical examination. On the other hand, exceptions to Profeta's law have occurred ; for example, in districts where syphilis is very common infants of syphilitic mothers commonly acquire syphilis, and instances have been recorded of infants born of such mothers developing primary sores on the head, the infection having been acquired during, or shortly after, birth. Matzenauer quotes the experiments of Uhlenhuth and Mulzer (1913¹) and others, which show that *S. pallida* introduced into the circulation of a pregnant rabbit can be found in the foetal circulation within five minutes. He quotes also Philipp, who has found that, although trypanosomes cannot pass the undamaged placenta, *S. pallida* can.

Gammeltoft (1929) thinks that infection of the foetus does not occur prior to the fourth or fifth month and that this view is supported by Thomsen's investigations confirmed later by Trichinese (1913). He does not subscribe to the view of Rietschel that infection is most apt to occur in the last month of pregnancy when the commencing separation of the placenta may give the spirochæte a direct channel to the foetal circulation.

Although nearly all workers believe that infection is always maternal, some think it is sometimes paternal. Thus Hochsinger (1929) is a firm believer in paternal transmission, and Almkvist (1929) recently published a paper in which he related cases in his experience of mothers of congenital syphilitics remaining free from all signs, serological and clinical, for numbers of years. The question can be decided only by the results of autopsy of such a mother coupled with proof that the offspring's infection was inherited and not acquired.

Instances of conveyance of infection by transfusion have been related by Feldman (1928), and the results of experimental work on animals show that in the primary and secondary stages of syphilis the blood has often been found infectious. In the primary and secondary stages, Uhlenhuth and Mulzer (1913²) found the blood infectious in a high proportion of cases, in the tertiary stage it was rarely so. In the latent stages they infected rabbits with blood from 2 out of 15 cases, one with an

infection of four years' standing, but in both these cases the patients had recently given birth to syphilitic infants and one a few days later showed a secondary rash. Frühwald also succeeded in infecting rabbits with blood of two latent cases, one with an infection a year old. Uhlenhuth and Mulzer (1913³) have also infected rabbits with milk of two women with no outward sign of syphilis, who had recently given birth to syphilitic infants, and Arzt and Kerl (1914) obtained the same result with milk from a woman infected five years previously. All the evidence goes to show, however, that the infectiousness of the blood usually dies out quickly after the first year, and McNamara (1925) has reported that he repeatedly transfused ten non-syphilitic patients from six suffering from late manifestations of syphilis without infecting the recipients.

SPEED OF INVASION AND MODES OF SPREAD IN THE TISSUES.

The speed of invasion of the deeper tissues in man is not known with any accuracy, but animal experiments indicate that it is rapid. Metchnikoff and Roux (1906¹) prevented infection of a man and a macaque monkey after experimental inoculation by rubbing 30 per cent. calomel ointment into the site an hour later. Of three macaques inoculated at the same time, one was treated with the ointment 20 hours later, but became infected, as did the remaining two not treated at all. It is interesting that the periods from inoculation to outbreak of the primary sore were 17 and 18 days in the two untreated monkeys, but 39 days in the monkey treated 20 hours after the inoculation. This, however, demonstrates only an inhibitory effect of the ointment on development of the primary lesion, not on the spread of the virus into the tissues. I have seen human cases in which a good antiseptic well applied within an hour of inoculation failed to prevent infection. Eicke and Schwabe (1921) reported an autopsy in the early primary stage which revealed infection of the mediastinal, mesenteric and other lymph glands. Kolle and Evers (1926) showed that in rabbits the spirochætes had reached the nearest lymph glands in half an hour after inoculation and in guinea-pigs in five minutes.

Modes of spread. The commonest route after inoculation is by the lymphatics, and thence through the blood-vessels to the rest of the body, but direct invasion of the blood-stream is not out of the question, and it seems reasonable to suppose, though it has never been proved, that spirochætes could travel by perineural lymph channels to the central nervous system.

SUSCEPTIBILITY OF EXPERIMENTAL ANIMALS.

In lower animals, after indecisive results by many workers in a large variety of animals from the year 1844, monkeys, especially the higher apes, were first decisively proved to be susceptible by Metchnikoff and Roux (1903), who cited previous experiments on these animals by Klebs in 1879, by Sperk in 1886, and by M. Nicolle in 1893, in some of which it seems probable that the disease was conveyed. Subsequently, valuable

work on several species of monkeys was carried out by Metchnikoff and Roux (1903 to 1906), Neisser with Baermann and Halberstädter (1906), Finger and Landsteiner (1905, 1906), and others. Monkeys have, however, now been superseded by other animals, especially rabbits and mice, and a detailed discussion of the earlier work will therefore be omitted.

The credit for first demonstrating the susceptibility of rabbits to syphilitic infection is usually given to Bertarelli, but Haensell is quoted by Levaditi and Roché (1909) as having produced keratitis and iritis by inoculation of gummatous material into the anterior chambers of rabbits, the incubation period being 25 days. Bertarelli (1906) produced similar results and demonstrated *S. pallida* between the layers of the infected corneas, and that by passage the virulence of the parasite for rabbits was increased. These results were confirmed by others, including Hoffmann and Brüning (1907), who also infected the cornea of a dog. Schucht is quoted by Neisser (1906) as having demonstrated generalization of the virus by infecting another rabbit with kidney tissue of a rabbit suffering from syphilitic keratitis. Recently, Grigoriew (1929) produced generalized syphilis by corneal inoculation and reported also that the infection was further passed from a doe thus infected to the unborn offspring. Parodi (1907) showed that a rabbit could be infected by inoculation into the testicle, and Levaditi and Yamanouchi (1908) by inoculation with syphilitic cornea under the prepuce. Uhlenhuth and Mulzer (1910¹ & ², 1911), who produced generalized syphilis by testicular and by intravenous and intracardial inoculation, Truffi (1909) and Nichols (1911, 1914) and Nichols and Hough (1913) were also early investigators on experimental syphilis in the rabbit, and the Truffi and Nichols strains of *S. pallida* are still used extensively in this work. The question of susceptibility of other animals will be discussed under 'Resistance' (p. 219).

It will be convenient now to give a description of the lesions which can occur in rabbits after experimental inoculation.

PATHOGENIC ACTION ON RABBITS.

The reaction of rabbits to syphilitic infection has been studied in considerable detail by Brown and Pearce, sometimes in collaboration with Witherbee.

Intratesticular Inoculation.

In Brown and Pearce's work on inoculation into the substance of the testicle, the inoculum contained an approximately constant dose of spirochætes, even so, not all animals reacted in the same way. The period elapsing from testicular inoculation to the first signs recognizable by external examination was usually 3 to 4 weeks, and in all cases between 2 and 6 weeks. Virus from active lesions produced a primary reaction more rapidly and usually a more intense one than did that from old, inactive, or regressing lesions.

The course of the testicular reaction was of two main types, but in both it was cyclical, recalling infections with blood spirochætes. There was an initial phase of more or less acute disturbance with swelling which was followed first by regression, and later by another flare-up usually of slighter intensity. This cycle might be repeated one or more times when the primary reaction was acute. In the acuter cases, after a comparatively short incubation period, the testicles swelled rapidly till the sixth day, when diffuse congestion with marked œdema of the scrotum was present : the scrotum and scrotal sac were filled with a gelatinous exudate, or with clear, or slightly blood-stained serous fluid which coagulated quickly on standing. The testicles also contained a similar exudate with some leucocytes and red blood-cells. On the seventh day regression commenced, congestion and œdema subsided rapidly and the size of the testicle decreased, till, in about a week, it was practically normal. After a quiescence of about 12 days the testicle again began to swell and continued to do so for another week. This was followed by another regression which was broken by a number of slight flare-ups.

In the more chronic type a circumscribed focus of induration gradually grew for 2 to 3 weeks until the testicle was large, smooth or slightly nodular, and very hard. Regression was more gradual and less complete, and was broken by one or more periods in which the testicle again slowly increased in size.

In practically all the animals the infection involved the whole organ, and often the scrotum. Although in many the process was apparently quite uniform, in others there was a finely granular condition of the testicle. In a third group there was distinctly greater reaction in certain portions of the testicular tissues than in others, and sometimes the outcome was a widespread necrosis of scrotal and testicular tissue.

Brown and Pearce describe certain variations and attribute them partly to inoculation with spirochætes whose vitality or infectivity had been materially reduced, and partly to peculiarities in the response of individual animals.

The spirochætes increased with the tissue reaction until the occurrence of the critical œdema, when they were found to have become immobile and collected in tangled masses. After this, parallel with the regression in the tissue reaction, they decreased in numbers until none, or only a few, degenerated or motionless specimens, could be found in fluid aspirated from the testicle. Shortly preceding the second increase of tissue reaction, the spirochætes again began to be found in increasing numbers and so on, the numbers of the parasites being roughly parallel with the increase and decrease of the tissue reaction.

Inoculation experiments with *S. pallida* taken from lesions during the critical œdema showed that at this time the virulence had considerably decreased. The incubation period was longer and the lesions were slower in development than when a much smaller dose of virus derived from active lesions was used. The local reaction persisted for a period which

was generally inversely proportional to the intensity of the reaction, active lesions rarely lasting longer than 2 to 4 months, and the less active ones persisting for 4 to 8 months.

Intradermal Inoculation.

When the inoculation was into the scrotal skin the incubation appeared to be shorter, viz. from 7 to 14 days, but this was due to the fact that changes in the scrotal tissues are more visible and can, therefore, be detected earlier. The earliest reaction was an oedematous swelling and congestion, with a growth of new vessels, or an infiltration with proliferation of fixed tissue cells. The reaction was either circumscribed, or diffuse and spreading, and the course was cyclical, like that in the testicle. The lesions were of two general types—a circumscribed granuloma, like the human chancre, or a diffuse infiltration analogous to secondary skin lesions in man, and in both there were a number of individual variations, and frequent and marked transformations. After a period varying from a few weeks to many months the lesions disappeared spontaneously.

Lesions Subsequent to the Primary.

In the case of circumscribed scrotal lesions, multiple foci of secondary reaction may develop locally subsequent to the regression or healing of the primary sore. Thus in one case with small circumscribed primary lesions at first nothing unusual happened. Then small, diffuse thickenings developed about the primary lesions, and small, thickened plaques occurred here and there in the scrotum. The primary lesions at the same time began to grow actively, the scrotum became thickened, and finally primary and secondary foci tended to fuse into a large granuloma, like an ordinary chancre. These changes coincided with the development of secondary lesions in the feet and legs.

Another form of local spread was perivascular, nodules developing along the course of the skin-vessels. In other cases local spread was manifested by discrete lesions which seemed to have no direct connection with the primary. Spread to inguinal glands with resulting adenopathy occurred as in human beings.

Metastatic Orchitis.

After healing of local lesions, whether spontaneous or resulting from constitutional treatment, the spirochætes in them might become active and the lesions recur, but it was rare for a recurrence to take place exactly at the site of a lesion which had healed completely. Besides recurrences in the scrotum it was common to find them in the testicle.

Invasion of Blood-stream, Glands and Other Tissues.

Pearce and Brown (1919) found that the blood was infective from a week or earlier after inoculation until regression of primary lesions occurred. They amputated the scrotum and testicle of 10 rabbits 48 hours after inoculation of the scrotum, and found that the virus had already

become generalized in all the animals. Thus, emulsions of excised lymph-glands inoculated into other animals infected them. Pearce and Brown (1922) demonstrated the presence of the virus in inguinal glands as early as 48 hours after scrotal inoculation. In rabbits with no active lesions the popliteal glands were found by the same method to be infective 3 to 25 months after infection. *S. pallida* could be demonstrated microscopically in only a small proportion of lymph glands. Nevertheless, the incubation period of the infection resulting from inoculation of glands with few spirochætes was only 7 to 10 days longer than that following an inoculation with material from an active testicular lesion containing numerous spirochætes. Pearce and Brown infer from this that the actual number of spirochætes is not nearly so important as their state of vitality or degree of infectivity.

Following on the metastatic orchitis and invasion of glands mentioned above, the disease in rabbits either becomes latent, or may become active in other structures. Amongst the earliest results of such activity are lesions of the bones, cartilages, tendons and synovial membranes (Brown, Pearce and Witherbee, 1921). The bones most commonly affected are those of the face, feet and legs, the lesions usually arising from the periosteum. Histologically they are syphilitic granulomata and lead to considerable necrosis, resulting in deformities such as saddle-nose, fractures and separation of epiphyses. Granulomata of tendons or tendon sheaths are seen occasionally, and changes in synovial membranes can be demonstrated microscopically.

Following on such lesions in point of time come lesions of the skin and then of the eye. Metastatic keratitis has been described by numerous workers since 1908, ciliary injection, conjunctivitis, keratitis and iritis by Brown and Pearce (1921), and choroiditis and chorioretinitis by Nichols (1914) and by Reasoner (1916), both of whom found that certain strains seemed particularly apt to cause eye lesions. Brown and Pearce noted, however, that affections of the eye might be frequent or rare with a given strain of spirochætes, whether they developed or not depending largely on the protection conferred by reactions in other tissues. Visceral lesions appear to be uncommon in syphilitic rabbits, but Brown and Pearce (1926) have described infiltrations of the myocardium similar to those described by Warthin (1914, 1916) in syphilitic myocarditis in man. They also (1920¹) demonstrated invasion of the central nervous system by infecting other rabbits with cerebrospinal fluid from one rabbit with early testicular infection and from two with actively progressing generalized lesions. Fluids from five rabbits with receding generalized lesions all failed to infect. In the last group particularly the fluid contained an increased number of cells, indicating an antecedent infection.

Brown and Pearce concluded that the disease in rabbits is not entirely comparable to either acquired or congenital syphilis in man, being a combination of the two, and in generalization bone lesions usually precede those of the skin. They suggested that the development of manifestations

of syphilis in rabbits was governed by two laws, 'the law of progression or sequence' and 'the law of inverse proportion'. The first is that in general, rabbit's tissues react in the order of chancre followed by lesions of bones, skin and eyes. The second is that the degree of a reaction in a given tissue depends on the degree of reaction in the preceding tissue attacked.

PATHOLOGICAL RESULTS IN MAN.

The period from invasion to the appearance of a visible lesion in man is usually about 30 days, with variations from 10 to 90 days.

Histological Changes at Sites of Inoculation.

The dominant histological features of a primary syphilitic lesion are the formation of new vessels in the early stages, infiltration with lymphocytes, plasma cells and then fibroblasts, and in old lesions the presence of some giant cells. The endothelium of the smaller vessels proliferates, their walls become thickened, and partial or complete occlusion results. Lastly, degeneration of the centre of discrete lesions and eventual fibrosis are constant in syphilitic lesions at all stages of the disease.

Spirochætes in primary lesions are distributed irregularly. They are scanty or absent in the central, degenerated area, and elsewhere are in clusters, chiefly in the lymph spaces and around the vessels, from which they penetrate into the surrounding connective tissue and between the epithelial cells. Levaditi and Manouélian (1905) demonstrated them also within blood-vessels. They were seen by Ehrmann (1906) in the perineural lymph spaces, and enclosed in fibroblasts, polynuclear leucocytes and endothelial cells. The spirochætes have been found in sections of the scars of primary lesions many years after the original infection. In active lesions it is easy to demonstrate their presence by scraping the margin and examining the juice expressed from the deeper parts.

Changes in the Regional Lymphatics and Glands.

From the site of inoculation the spirochætes spread along the lymphatics, with reaction which interferes more or less with the lymphatic drainage, and produces corresponding cedema, sometimes very pronounced. Occasionally this reaction is so marked that the lymphatic vessels can be felt as subcutaneous beaded cords in the dorsum of the penis.

In the regional lymph glands spirochætes are distributed unevenly in the lymph spaces, the walls of the blood-vessels and the trabeculæ. The tissue reaction is commonly that seen in any other chronic adenitis, viz. marked overgrowth of the stroma, with thickening of the trabeculæ, and great increase of endothelial cells, some of which may show more than one nucleus. Fibroblasts and round cells are seen around the glands, which become consistently increased in size.

Michelson (1929), in an examination of inguinal glands from patients with early untreated syphilis, found that some had a markedly tuberculoid

structure such as is not commonly found in the primary and secondary stages, but occurs in old untreated chancres, lichen syphiliticus and gumma. Plasma cells were sometimes present in sections showing the ordinary changes of chronic inflammation, but in none were they so abundant as in a syphilitic papule examined at the same time. Two types of tuberculoid tissue were found, one consisting almost entirely of epithelioid cells closely packed into lobules, in the other nodules very like miliary tubercles were scattered throughout the gland, or in adjacent areas, especially near to the hilum. Occasionally several small foci had coalesced as in tubercular adenitis, but without caseation. *S. pallida* was found in five of the eight glands presenting these appearances. It seems possible that the defensive role of lymph glands may account for the comparative scarcity of microscopically demonstrable *S. pallida* in the infected glands of syphilitic rabbits (see p. 203). In human beings, however, it is usually easy to demonstrate the presence of the spirochæte in regional lymph glands by microscopical examination of the aspirated gland juice.

Spread to Other Tissues.

As already mentioned, before the outbreak of the primary lesion, *S. pallida* has spread throughout the body. Although there is probably no part of the body in which syphilitic lesions have not been described, the tissues are not equally active in response. For example, in man (unlike rabbits), after the glands, the skin and the mucous membranes of epiblastic origin commonly show earlier and more extensive reactions than does the parenchyma of the central nervous system. Also, although *S. pallida* has a predilection for perivascular lymph spaces, and in all lesions disease of the walls of small vessels is a dominant feature, symptoms due to disease of medium-sized and larger vessels are not common in early stages, but, like diffuse myocarditis, are events to be expected in the later years of the infection. It seems possible that the laws of progression or sequence and of inverse proportion formulated by Brown and Pearce (p. 204) for the course of syphilis in rabbits may be applicable to human beings. That some human tissues can be naturally unresponsive is seen in the infrequency with which certain organs are affected and possibly in the differences between males and females in regard to the organs commonly found to be diseased at autopsy, to which Warthin (1928) has drawn attention. In the average case the glands throughout the body react before the skin and mucous membranes, and general adenopathy is probably the most persistent macroscopic feature of syphilis.

The Skin and Mucous Membranes.

In the first skin reaction, the roseola, which follows the primary lesion in a few weeks, the microscopic changes are only slight and are centred round the vessels of the papillæ and corium, which are dilated and infiltrated with lymphocytes and plasma cells. The syphilitic papule,

which commonly forms in the centre of a macule, presents much more characteristic changes. Around the vessels is an infiltration of round cells amongst which are numerous plasma cells. Thickening of vessel walls, with partial or complete occlusion, is a prominent feature. There is proliferation of epidermis, especially between the papillæ, which raises the lesion above the general surface of the skin. An exaggeration of the hyperplasia is seen in the broad condyloma. Degeneration of the central portions of the lesion is seen in the mildest form in scaling, in moderate forms in necrosis of the centre or of the whole papule, and in the rare malignant syphilides in more or less ulceration. Resolution is considerably slower than in the case of the roseola and is often accompanied by pigmentation of the site of the lesion, attributed by Ehrmann to melanoblasts. Besides this, changes in the distribution of the pigment (especially on the neck) causing syphilitic leucoderma are occasional residua, the cause of which is not clear. Secondary lesions in the mucous membranes have essentially the same histological characteristics as those of the papule.

In most secondary lesions, except perhaps the roseolar macule, it is quite easy to demonstrate *S. pallida* by scraping the lesion and examining the serum which exudes. In the case of the roseola, Levaditi and Petresco (1905) found that the organism could be obtained by applying a blister to the macule and afterward lightly scraping the raw area.

The skin is more commonly affected in the later stages than any other part of the body, the characteristic lesion being a small gumma, the histology of which in its commencement is essentially that of the primary lesion. The tissue reaction is, however, more pronounced in relation to the numbers of spirochætes, which are rarely found and then only in the periphery of the lesion. Obliteration of vessels is more extensive, giant cells are commoner, the centre of the lesion is more likely to become necrosed, and the formation of connective tissue in the periphery of the lesion is more certain and definite.

Subcutaneous Tissues, Muscles, Bones and Joints.

In the subcutaneous tissues and muscles in the early stages it is uncommon to find lesions, but occasionally there is a reaction in the bones in the secondary stage in the form of a mild periostitis, and synovitis is not uncommon. In the later stages the reaction of all these tissues is in the form of diffuse infiltration, or of discrete gumma which is histologically the same as gumma elsewhere. In bones mucoid transformation of the tissue is a commoner event than in the skin, muscles and subcutaneous tissues. It is usually said that the gumma is the characteristic lesion of the later stages of syphilis, but Warthin (1918) insists that gumma in the internal organs is rare in comparison with diffuse infiltration. Diffuse infiltration is by no means uncommon elsewhere in the body, and a more careful search, such as Warthin conducted in the organs, might show it to be the predominant lesion.

Viscera.

Few opportunities have occurred of studying the histological changes in the viscera in early acquired syphilis, and main reliance must be placed on examinations of the organs of infants dead of congenital syphilis. De Souza Campos (1923) examined the *kidneys* of a number of syphilitic infants, 39 premature and 30 others, and found changes in the cortex, but the medulla was usually little affected. A cellular infiltration was present around the cortical blood-vessels consisting of neutrophile and eosinophile myelocytes, normoblasts and megalocaryocytes, which separated the tubules, and spirochaetes were numerous in this region. Warthin (1922) also found *S. pallida* more abundantly in the kidney in congenital syphilis than even in the liver. Various workers have demonstrated *S. pallida* in the urine of secondary syphilitics, and the occasional occurrence of albuminuria is evidence of invasion of the kidney in the early stages of the acquired disease. In almost all later cases examined Warthin (1918) found chronic passive congestion, atrophy, infarctions and local and diffuse inflammation and chronic parenchymatous nephritis.

Histological evidence of lesions of the *liver* in the early stages of acquired syphilis is scarce, but the rare occurrence of acute yellow atrophy in the early exanthematous and later stages of syphilis has been attributed to this disease. Thus Mayer (1921) investigated 25 cases of acute yellow atrophy not due to phosphorus or other poisons. There was definite evidence of syphilis in 15, probable in 5, none in 2, and 3 were pregnant. He thought syphilis a constant factor in such cases, but admitted the necessity of another factor, possibly biliary infection. McIntosh and Fildes (1914) regarded acute yellow atrophy as due to a diffuse invasion of the liver cells, which cannot respond to the toxin as do connective tissue cells, but, like the brain cells in general paresis, degenerate and die. At the other extreme is the circumscribed focus commencing as a peripylephlebitis and resulting in a gumma. Between the two extremes, as seen in congenital syphilis, are all grades of diffusion, with interstitial reaction of the ordinary lymphocyte and plasma cell type and also invasion of the liver cells resulting in the formation of cell masses containing multiple nuclei and ultimate degeneration; in fact a combination of parenchymatous and of so-called gummatous hepatitis. The end result of the interstitial process is fibrosis, contraction of fibrous tissue and corresponding interference with the functions of the organ. Warthin (1918) constantly found changes in the *adrenals*, which in most cases were slight, but in three there was great atrophy with signs of adrenal deficiency during life.

In the *pancreas* of all old syphilitics he found atrophic and fibrotic changes, with scattered plasma cell infiltrations and eventual fibrosis. In all diabetic cases but two, island fibrosis was marked and characteristic, chiefly large hyperchromatic cells round the border of the hyaline fibroid island. Spirochaetes were demonstrated in these areas in two diabetic cases.

Syphilitic lesions have been shown at one time or another in all other glands of the endocrine system.

Genital Organs.

Testis. In the testis, although the occurrence of gross lesions is usually deferred until a later stage, evidence of invasion is afforded by the demonstration of *S. pallida* in the semen of secondary syphilitics by inoculation of rabbits. Finger and Landsteiner (1906) were among the first to infect monkeys with semen from two cases of syphilis, and Finger (1926) has related what was probably a case of seminal infection from husband to wife. Noegerrath and Staehelin (1905) found *S. pallida* in the semen in three cases of secondary syphilis. In the latent stages, Kertesz (1928) demonstrated *S. pallida* in the semen of 5 out of 20 cases by inoculation into rabbit's corneas.

In latent syphilis histological changes are found in the testes as consistently as in any other tissue. Warthin (1918) saw syphilitic changes in the testes of all of 300 cases in which there was evidence of a syphilitic infection and in 75 of them he found *S. pallida*. Saleeby (1926) examined 40 testes from syphilitic cadavera. None of the testes presented gross appearances, but 23 showed histologically syphilitic infiltration around vessels and fibroblastic proliferation in the stroma. In some cases hyaline changes and fibrosis were strongly marked. Some tubules showed moderate atrophy, the basement membrane was thickened, and as a rule there was diminished spermatogenesis. *S. pallida* was demonstrated in only one case, a still-born.

Histologically proved syphilitic lesions of the *prostate* have rarely been reported. Thompson (1920¹) found only 24 reports in the literature, in all but one of which the diagnosis was clinical. Starry (1924) reported a case, with histological details, which included the finding of *S. pallida*, in a man of 73.

Uterus, tubes and ovaries. Although syphilitic changes have been found in the uterus early in the disease, Warthin (1929) states that he has never found changes, or *S. pallida*, in the adult ovaries or tubes. On the other hand, Levaditi and Sauvage (1906) found *S. pallida* in the ovary and even in the ova of a syphilitic infant, and Simmonds (1906) found it in the ovary of an infant. v. Kubinyi and Johan (1922) have described a gumma containing *S. pallida* in the ovary of an adult who was apparently a congenital syphilitic. Menninger (1928) reviewed the literature, which showed the comparative rarity of syphilitic changes in the ovaries, and related the case of a thirteen-month old infant in which he had found *S. pallida* and characteristic changes.

Bladder.

Syphilis of the bladder is generally thought to be rare, but Thompson (1920²) considered that it was commoner than is usually thought to be the case. Of 58 undoubted cases discovered in the literature, only 7 were recorded as in the first year, but in 2 others the stage must have been early.

Stomach and Intestines.

Changes in the stomach and intestines do not appear to have been noted in early stages, but various cases of gummata in later stages have been reported. Eusterman (1918), in a review of the subject, remarked on the rarity with which the condition was found by the histologist as compared with the number of times it had been diagnosed clinically. He found that only a dozen or more of the cases which had been diagnosed clinically had been verified at autopsy. Gatewood and Kolodny (1923) related a case of fibrosis of the stomach and ulceration of the last 120 cm. of the ileum and of the large bowel which they considered on histological grounds to be syphilitic. McNee (1922) published a detailed account of the histology of a perforated gumma of the stomach in which he found *S. pallida* in great numbers. Welch (1928), apparently ignoring the description of the tissue changes, criticized this case because spirochætes were found only at the surface where necrosis had occurred, and also pointed out that elastic fibrils may look very like spirochætes.

Lungs.

Although syphilis of the lungs is a common enough finding in congenital cases, in acquired syphilis the reports as to its frequency (in gross forms at any rate) vary, but most agree as to the comparative rarity with which it has been proved histologically. Carrera (1920) quotes various writers who have mentioned the rarity of lung lesions in syphilis. Thus, there was no specimen in 13,000 in the Army Medical Museum, Washington, in 1905 ; only 10 in London museums in 1895 ; only 2 in 6,000 autopsies in Chicago ; and so on. Compared with cases found at autopsy, those discovered clinically are numerous. As a result of his own study, Carrera found that 12 out of the 152 cases of syphilis showed syphilitic changes in the lung, viz., 3 gumma, 2 peribronchitis with arteritis, 4 fibrosis with arteritis and 3 arteritis. Warthin (1929), commenting on the difficulty of diagnosing pulmonary syphilis histologically, said that he had not seen more than 15 cases which had been proved.

Cardiovascular System.

The cardiovascular system is by far the most important of those attacked by syphilis and, with the testes and central nervous system, the most frequently involved in males. ✓

The following description of the changes is based on reports by Warthin (1916, 1918, 1926, 1929). While gumma is relatively rare, diffuse infiltration can be found in practically every myocardium of a male syphilitic. The essential lesion is a diffuse, patchy, interstitial myocarditis, the infiltration following the vessels between the muscle fibres. It is often very slight and not visible to the naked eye, the cells being then in single file. They consist of lymphocytes, monocytes, plasma cells and large epithelioid fibroblasts ; polynuclears are not often seen. Commonly the infiltrates are in the septum and the anterior and posterior walls of the left ventricle rather than the right, and are not sharply demarcated. An

increase of nuclei just under the endocardium is very characteristic. Marked sclerosis of coronary arteries was rather rare in Warthin's cases, and thrombosis was not seen, but small terminal arterioles and capillaries were often obliterated. The muscle fibres between the infiltrations are often hypertrophied. Eventually a patchy fibrosis results and the muscles involved become atrophic and fatty; the fibrosis may become hyaline.

In five cases of congenital syphilis (1916) he found elevated myxoma-like nodules, 5 to 10 mm. in diameter, occupying the anterior wall of the left ventricle. They consisted of oedematous gelatinous connective tissue with remains of atrophic muscle-fibres, and contained *S. pallida* in large numbers.

Acute exacerbations of latent lesions may occur and are characterized by polynuclear infiltrations in which spirochætes are seen in large numbers. Warthin (1926) has drawn particular attention to this reaction with polymorphonuclear cells, which he finds in every way identical with the polymorphic reactions of the critical phases of animal syphilis. He regards it as due to an active exacerbation of the syphilitic infection, since spirochætes are present in very large numbers in the infiltrations. He reported eight cases of rapid death in which he found it particularly well marked in conjunction with the lymphocytic-fibrotic changes resulting from the chronic action of *S. pallida* on the heart tissues.

In the fibroid areas dilatation of existing capillaries, or new-formed capillaries, are often seen as large blood spaces, with walls composed practically only of lining endothelium. No case of mitral involvement was seen, but all showed involvement of the aorta. Usually at autopsy the heart of the latent syphilitic shows dilatation of the left and sometimes of both ventricles; aneurysm of the wall is a rare occurrence. The endocardium is thickened, and a cross section of the heart shows irregular areas of fibrosis, especially of the left ventricle towards the apex.

S. pallida was found more easily than in any other organ or tissue, sometimes lying between muscle-fibres which appeared to be normal. The method employed was the Warthin-Starry one (1921), by which, if syphilitic changes are seen in a given section, the next section may be stained for spirochætes. From Warthin's description of the histological changes in man, and Brown and Pearce's of those in rabbits, the usual process in latent syphilis appears to be one of repeated mild exacerbations of activity of *S. pallida*, to which the response is a monocytic infiltration that restores the balance against the spirochæte, but at the expense of more or less fibrosis with corresponding loss of function. Occasionally the balance is on the side of *S. pallida* so that it can multiply rapidly, the response then being a polynuclear infiltrate.

Warthin has seen active lesions in the aorta of every male and nearly every female syphilitic, though they may be only microscopic. The lesion is essentially an obliterating arteritis of the *vasa vasorum*, and the muscular layer of the aorta is not affected primarily; infiltrations are more marked in the adventitia. The result is infarctions in the inner layer

of the media and in the intima, with resulting degeneration of the muscle and of the elastic tissue. Cases which go on to aneurysm and aortic insufficiency are in the minority. The valvular lesion is due to involvement of the *vasa vasorum* of these structures, and aneurysm is usually due to a localized development of miliary gummata around the *vasa vasorum* as they course through the media. Acute exacerbations of aortic disease may occur, and are characterized by polynuclear infiltrations in which spirochætes are seen in large numbers. In ordinary lesions spirochætes are seen in small groups around the *vasa*.

The para-aortic vessels are a favourite, sometimes the only, site of vascular lesions in latent syphilis. The changes leading to aneurysm of other large arteries are similar to those in the aorta.

Syphilitic affections of the walls of veins are infrequent. Morrow and Epstein (1928) discovered records in the literature of 39 cases in the early stages and 6 in the later. In the early cases superficial veins were chiefly affected and in late ones the deeper veins. Marshall (1925) recorded a case of elephantiasis of the scrotum which he attributed to syphilitic arteritis and phlebitis of the local vessels.

Blood Changes.

In the early stages of acquired syphilis there is a moderate fall in the number of red cells and a more pronounced reduction in the hæmoglobin—these changes being most marked at the height of the secondary exanthemata. More severe degrees of anæmia have been reported (see Andrewes, 1908), even with a blood picture of pernicious anæmia. Severe anæmia is rarer in acquired syphilis than in congenital syphilis, possibly because here the liver is more profoundly damaged. Eason (1921), in secondary syphilis, records cases of severe anæmia, and in tertiary syphilis recognizes a considerable variety of blood changes which are attributable in some measure to the disease, such as simple anæmia, with and without splenic enlargement, cases of polycythæmia, and those with the blood picture of pernicious anæmia, both usually with enlarged spleen. There is also hæmolytic anæmia of paroxysmal hæmoglobinuria. Browning and Watson (1913) found syphilis present in all their collection of 59 cases of paroxysmal hæmoglobinuria. Eason also describes cases of chronic splenomegaly in late syphilis, with the features of Banti's disease except that liver and spleen were tender.

More striking changes are seen in the leucocytes, and Hazen's (1913) study of a large number of cases in different stages showed that in the primary and secondary stages there is a moderate leucocytosis up to 20,000. In untreated cases the increase was mainly attributable to polymorphonuclears and in treated ones to lymphocytes. Under treatment the lymphocytosis might reach 65 per cent. and persist for months. Severe cases of secondary syphilis in Hazen's series usually showed a higher total, due to neutrophiles, than did milder ones, and all the secondary cases which did badly under treatment showed a relatively high proportion

of polynuclears and low one of lymphocytes. Tertiary cases rarely showed any increase, and the differential counts were not far from normal. Marked general enlargement of glands was not necessarily associated with lymphocytosis. The association of a neutrophile leucocytosis with the acuter stages of the disease is interesting in connection with Warthin's finding (p. 210) that in acute exacerbations the infiltrate is composed of polynuclear cells with numbers of spirochætes.

Changes in the blood-serum will be reviewed later (p. 229).

The Central Nervous System.

/ It is generally admitted that the central nervous system is invaded early. Besides invasion by the blood-stream, spirochætes may possibly reach the parenchyma of the nervous system by the perineural lymph channels, for Orr and Rows (1904) confirmed the work of Homén which demonstrated the existence of an afferent perineural lymph stream, and Ehrmann (1906) demonstrated *S. pallida* in the perineural lymph spaces in primary lesions.

Early evidence of reaction by the tissues is seen in changes in the cerebrospinal fluid to which Ravaut (1903) first drew attention when he reported that in a high proportion (72 per cent.) of secondary syphilitics with various lesions he had found a pleocytosis and other pathological changes (these will be discussed in more detail under 'Diagnosis', p. 264). Cases of syphilis which show pathological changes in the spinal fluid are much more numerous than those with clinical signs of nervous disease; about one-third have pathological fluid at one time or another, and the changes may commence very early. Fildes, Parnell and Maitland (1918) found in 71 cases with chancre but negative serum reactions, 9 with a doubtful, and 2 with a definite pleocytosis. In 168 cases with chancre, adenopathy and positive serum reactions, but no sign or history of generalized symptoms, they found 17 with a doubtful, and 15 with a definite, pleocytosis. Wile and Hasley (1921), in 221 primary cases, of which 106 gave negative serum reactions, found 11 with an increase of one or two cells above their standard of 8 per c.mm. and 12 with a definite pleocytosis, and 49 in all had either pleocytosis, increase of albumin and globulin, or a positive Wassermann reaction. In Fildes, Parnell and Maitland's series of 624 unselected cases of syphilis the incidence of those with pathological fluid reached 29 per cent. in cases with generalized signs or history of such and examined from three to six months after infection. In similar cases examined more than six months after infection it was 17 per cent., but in cases without signs other than a positive serum reaction more than six months after infection it was 35 per cent.

The presence of *S. pallida* in the cerebrospinal fluid of a secondary case of syphilis was demonstrated by Hoffmann (1906) by inoculation into animals, by Nichols and Hough (1913), who infected a rabbit with fluid from a case of cerebrospinal syphilis, and by Wile (1917) with fluids from cerebrospinal syphilis and general paresis.

Direct demonstration of *S. pallida* in the cerebrospinal fluid is not easy. Sézary and Paillard (1910) were the first to find them by the dark-ground illumination method, while Wile and Kirchner (1923) found them by a special method in 12 out of 118 specimens. Schönfeld and Krey (1927), after 500 failures to discover *S. pallida* in cerebrospinal fluid by the dark-ground method, adopted the Wile and Kirchner method and found the organism in 8 out of 17 specimens. Three of the eight fluids gave positive reactions with ordinary tests and five gave negative. The method was to coagulate the proteins of the fluid with 96 per cent. alcohol, centrifugalize, replace the alcohol with 10 per cent. formalin for 24 hours, separate the deposit, recentrifuge and then embed and section the deposit by the Warthin-Starry method for demonstration of *S. pallida* in tissues.

The direct relation of *S. pallida* to the lesions of parenchymatous neurosyphilis was first demonstrated by Noguchi and Moore (1913), who found them in 12 out of 70 specimens. Mott (1914¹) reported that he had found the organism 66 times in 100 brains of general paretics. Cases with marked cortical wasting yielded far less satisfactory results than did recent cases. Forster and Tomaszewski (1913) first demonstrated the presence of *S. pallida* in the brains of living paretics, the specimens being obtained by the Neisser-Pollak method of puncture. They reported success in 27 out of 61 cases, the spirochætes being abundant in 11 and very scanty in 16.

All workers agree that *S. pallida* is found principally in the cortex. The spirochætes are usually in clusters, though sometimes disseminated, in close relation with, and sometimes within, the pyramidal cells. They are also found in the periphery of the blood-vessels. They appear to be more abundant in cases of juvenile paresis.

In the spinal cords of tabetics only one discovery of *S. pallida* has been reported, by Noguchi (1913), who found it in the dorsal column once in 12 cases.

The changes in the tissues of the central nervous system affect both the membranes and the parenchyma, but the latter is rarely affected without the former. The changes in the membranes do not differ essentially from those in other parts of the body, but certain features, e.g. aneurysm and obliterating arteritis, are more prominent. In early cases of syphilitic meningitis, e.g. three months after infection, there is a diffuse infiltration affecting more particularly the pia-arachnoid, which is considerably thickened and oedematous, especially in the interpeduncular space and at the optic chiasma. The infiltration involves also the cranial and spinal nerves and follows the pia mater into the substance of the brain and cord, with consequent damage of nerve tissue; miliary and sometimes larger gummata may be mingled with the diffuse infiltration. The process is associated with obliterating arteritis, so that partial or complete occlusion of vessels, either by surrounding pressure or by thickening of the coats, is a common event. Aneurysmal dilatation, particularly of the vessels entering the circle of Willis, with possible rupture is another sequel.

The fact that many cases show changes in the spinal fluid without nervous symptoms justifies a belief that in a large proportion of cases the reaction of the meninges is by no means so severe as sketched above. Further, the greater frequency in the secondary stage of symptoms indicative of a diffuse meningitis and nerve-root irritation, than of symptoms pointing to paresis or paralysis of one particular nerve or group of nerves, suggest that nerve tissue probably presents considerable resistance to the process. Indeed, far more damage may be found *post mortem* than the symptoms during life suggested.

In the later stages of syphilis the granulomatous process tends, as in other tissues, to be limited to comparatively small areas, but the individual lesion to be larger.

In general paresis the outstanding change is degeneration of the neurones with great increase of neuroglia. It is usually most pronounced in the fronto-central convolutions, but progressively involves more and more areas, until in an advanced case the cells of the basal ganglia, the spinal cord and the cerebellum show atrophic changes. Associated usually with the neuronc decay and the neuroglial proliferation is considerable thickening of the pia-arachnoid, with perivascular infiltration, as in ordinary meningitis.

The change in tabes appears to commence at the point of entry into the cord of exogenous nerve-fibres which have their trophic centres in the posterior root ganglia and form the cores of the posterior nerve-roots. Later, as is well known, the process extends to the endogenous fibres in the posterior columns and often elsewhere in the parenchyma of the cord. The nature of the process leading to this degeneration is still in dispute. Meningeal changes, though often present, are not constant, so that the theory of Redlich and Obersteiner attributing the degeneration in tabes to meningeal fibrosis constricting posterior nerve-root fibres is insufficient. Changes in the ganglion cells can hardly originate the process, for they are not always degenerated. Orr and Rows (1904) have confirmed Homén's (1902) finding that the perineural lymph stream is cord-ward in the sensory nerves, and have shown that the degenerative changes always commence at the point of entry of the root fibres into the cord, where possibly they become more vulnerable to toxins by losing their neurilemma.

McIntosh and Fildes (1914) have advanced the argument that parenchymatous neurosyphilis does not differ essentially from parenchymatous syphilis elsewhere, in the liver and kidneys, for example, though in these organs the parenchymatous degeneration does not stand out so clearly from the interstitial process as in the nervous system, where there is a clear-cut barrier between the pial sheath and the nerve tissue. As they conceive the process, the tissues, having become allergic by long interaction between themselves and the virus, respond to an exacerbation of the activity of *S. pallida* each in its own way, the supporting and nutritional structures by proliferation, the parenchymatous by cloudy

swelling, followed by degeneration. The reason of *S. pallida* invading the parenchyma in some cases and of remaining more or less confined to the interstitial tissues in most is by no means clear.

Certain peculiarities of parenchymatous neurosyphilis, particularly the frequent absence of any history of cutaneous lesions, have suggested a theory propounded by Morel-Lavallée in 1889, and favoured since by Nonne and Levaditi and Marie and others, that it is due to a neurotropic, as distinct from the ordinary dermatropic, type of *S. pallida*. From the clinical side numerous instances of persons infected from a particular source who developed tabes or general paresis have been advanced in favour of the theory. Moore and Keidel (1921) and Moore and Kemp (1923) have made clinical and laboratory studies of 113 conjugal partners (94 women and 19 men) of 111 neurosyphilitics and found that the incidence of neurosyphilis in the partners of parenchymatous neurosyphilitics was more than twice that of the partners of non-parenchymatous neurosyphilitics. In cases of asymptomatic neurosyphilis, i.e. diagnosed only by changes in cerebrospinal fluid, the fluids of partners of parenchymatous neurosyphilitics were of the kind (authors' Group 3) usually present in paretics, while the fluids of partners of non-parenchymatous neurosyphilitics were of the type (authors' Group 2) which the authors regard as the precursor of diffuse cerebrospinal syphilis. The histories of the syphilitic partners of parenchymatous neurosyphilitics showed that only one-third had had obvious lesions outside the nervous system, while more than two-thirds of the partners of the non-parenchymatous neurosyphilitics had had such lesions. An analysis of the figures collected by other workers did not, however, show such a striking difference between the two groups—not more than about 8 per cent. Moore and Kemp, although remarking that their statistics were significant, consider that, from the clinical standpoint, the question of neurotropism cannot be solved. Sicard (1925), who is no believer in neurotropic strains, reported two cases of general paresis infected by persons who showed no sign of neurosyphilis.

Experimental work to determine by morphological appearances and the reaction of experimental animals to inoculation with *S. pallida* from paretics which has been carried out by Noguchi (1912), Reasoner (1916), Levaditi and Marie (1919) and Plaut and Mulzer (1922) does not seem to have contributed anything towards an answer to the question of the existence of a neurotropic as distinct from a dermatropic strain.

If a neurotropic strain does exist, it must naturally be to some extent dermatropic since the neurosyphilitic is infectious. In the paper by Moore and Kemp which is quoted above, instances are given of infection of wives by neurosyphilitic husbands who had contracted syphilis respectively 13 and 10½ years previously. Numerous workers do not believe in the existence of a specially neurotropic strain, and Sézary (1926) points to the well-known rarity of parenchymatous neurosyphilis in coloured races, yet whites infected by them develop tabes and paresis,

and the incidence of pleocytosis in the cerebrospinal fluid of syphilitic Arabs is the same as in syphilitic Europeans.

Family predisposition may be an important factor in the development of neurosyphilis. Moore and Keidel (1923) and Moore and Kemp (1923) note the frequency of neurosyphilis in certain families, and Kemp and Poole (1925) observed that neurosyphilis among the children of 20 neurosyphilitic families was three to eight times more frequent than among the children of 20 non-neurosyphilitic families.

Gennerich (1922) and others regard allergy resulting from interaction between the skin and the spirochæte as the important factor. If this is weak, by reason of natural non-reactivity of the skin or resulting from treatment, there is correspondingly greater activity of *S. pallida* in the internal organs. Montpelier (1926) and others suggest that *S. pallida* derived from persons who have been treated with specific remedies does not excite in the second host the strong skin reactions which result in formation of protective bodies. According to Gennerich, the first consequence of a weakened allergy so far as the nervous system is concerned is a chronic meningitis, leading to weakening of the pia mater and eventually to penetration of the cerebrospinal fluid into the parenchyma, with drenching of the nerve tissue, which is assumed to destroy its natural resistance. In support of this theory, Gennerich instances, *inter alia*, the increased sensitivity (pain, hyperæsthesia, &c.) of the nerve-roots of tabetics to neosalvarsan injected intrathecally, compared with cases of cerebral disease with intact cord membranes, due, he says, to the greater permeability of the membranes. He explains the localization of parenchymatous neurosyphilis on hydromechanical grounds, and Mott (1914) observed that, in general paresis, the area most affected is that in which the anatomical conditions of the blood-supply favour back pressure.

Recognition of Syphilis in a Macerated Fœtus.

The changes in the liver, kidney and other organs of the congenital syphilitic, considered above, may not be of much value for diagnosis in a fœtus expelled some time after death. In such cases the following may be of value.

The placenta. The characteristic change in the placenta is a hyperplasia of the chorionic villi, with increase in the collagen fibres and of the cells, affecting the whole placenta or only sections, and healthy and affected villi may be seen side by side. The vascularity of the affected villi is greatly diminished, which is commonly attributed to perivascular infiltration of the vessels of the villi, but this was not found by Eardley Holland (1922), who attributed the avascularity to failure of the terminal villi to become vascularized in the first instance. An increase in the weight of the placenta relatively to that of the fœtus is commonly stated to be a useful sign in the diagnosis of a syphilitic placenta, but Holland does not believe that it is of any great value, for in his series heaviness was not constant in syphilitic placentas, and a certain number of the

non-syphilitic were heavy. Similarly, Holland does not attach any importance to such appearances as voluminous cotyledons, deep sulci, pale placental tissue, grey and greasy maternal surface, fibrous areas, unusual softness and friability, which are often said to be characteristic of the syphilitic placenta. Spirochaetes have not often been found in syphilitic placentas, but this is attributable to the lengthy search required.

The long bones. The important changes at the junction of the epiphysis with the diaphysis and in the epiphysis which Wegner termed 'osteochondritis syphilitica' are of great diagnostic value because they give rise to irregularities in ossification, signs of which are visible by radiographic examination. These changes were described by Turnbull in Eardley Holland's Report (1922, p. 41). Turnbull summarizes the changes as 'deepening of the zone of provisional calcification; irregularity of the line of junction between the epiphysis and the diaphysis; multiple zone of provisional calcification; enlargement of the chondral vascular canals; fibrosis in the marrow of the metaphysis or of the canals of the epiphyseal cartilage'. The changes are more intense in some bones than others, the femur, tibia, humerus and ribs being sites of election.

The liver is usually, though not invariably, heavy in relation to the weight of the foetus.

The spleen is usually also heavy, and abnormal weight of both liver and spleen, though not pathognomonic, is very suggestive.

REFERENCES.

- ALMKVIST, J., 1929, *Wien. klin. Wschr.*, **42**, 97.
 ANDREWES, F. W., 1908, cited from *A System of Syphilis*, by D'ARCY POWER & MURPHY, London (Oxford Med. Pub.), **1**, 123.
 ARZT, L. & KERL, W., 1914, *Wien. klin. Wschr.*, **27**, 785.
 BERTARELLI, E., 1906, *Zbl. Bakt.*, Abt. I, Orig., **41**, 320.
 BROWN, W. H. & PEARCE, L., 1920¹, *Arch. Derm. Syph.*, Wien, **2**, 635; 1920², *J. Exp. Med.*, **31**, 475, 709, 729, 749; 1921, *ibid.*, **34**, 167; 1924, *ibid.*, **39**, 645; 1926, *ibid.*, **43**, 501.
 BROWN, W. H., PEARCE, L. & WITHERBEE, W. D., 1921, *J. Exp. Med.*, **33**, 495, 515, 525.
 BROWNING, C. H. & WATSON, H. F., 1913, *Int. Clin.*, **2**, xxiii, 111.
 BUSCHKE, A. & FISCHER, W., 1906, *Berl. klin. Wschr.*, **43**, 383.
 CARRERA, J. L., 1920, *Amer. J. Syph.*, **4**, 1.
 EASON, J., 1921, *Brit. Med. J.*, ii, 186.
 EICKE & SCHWABE, E., 1921, *Münch. med. Wschr.*, **68**, 671.
 EHRLMANN, S., 1906, *Deuts. med. Wschr.*, **32**, 1115.
 EUSTERMAN, G. B., 1918, *Amer. J. Syph.*, **2**, 205.
 FELDMAN, V. J., 1928, *Arch. Derm. Syph.*, Wien, **18**, 380.
 FILDES, P., PARNELL, R. J. G. & MAITLAND, H. B., 1918, *Brain*, **41**, 255.
 FINGER, E., 1926, *Wien. klin. Wschr.*, **39** (Sonderbeilage).
 FINGER, E. & LANDSTEINER, K., 1905, *SitzBer. Akad. Wiss.*, Wien, **114**, Abt. 3, 497; 1906, *ibid.*, **115**, 179.
 FORSTER, E. & TOMASZEWSKI, E., 1913, *Deuts. med. Wschr.*, **39**, 1237.
 FRÜHWALD, R., 1914, *Derm. Wschr.*, **50**, 1319.
 GAMMELTOFT, S. A., 1929, *Amer. J. Syph.*, **13**, 194.
 GATEWOOD, W. E. & KOLODNY, A., 1923, *Amer. J. Syph.*, **7**, 648.
 GENNERICH, W., 1922, *Münch. med. Wschr.*, **69**, 922.
 GRIGORIEW, P., 1929, *Derm. Wschr.*, **89**, 1122.

- HAENSELL, *Graefes Arch.*, **27**, cited 1909 from LEVADITI & ROCHÉ, *La Syphilis* Paris (Masson), p. 33.
- HAZEN, H. H., 1913, *J. Cutan. Dis.*, **31**, 618.
- HOCHSINGER, K., 1929, *Wien. klin. Wschr.*, **42**, 43.
- HOFFMANN, E., 1906, *Derm. Z.*, **13**, 561.
- HOFFMANN, E. & BRÜNING, W., 1907, *Deuts. med. Wschr.*, **33**, 553.
- HOLLAND, E., 1922, *The Causation of Fœtal Death*, Rep. Publ. Hlth. Med. Subj., Lond., No. 7.
- HOMÉN, 1902, cited from Orr & Rows (1904).
- KEMP, J. E. & POOLE, A. K., 1925, *J. Amer. Med. Ass.*, **84**, 1395.
- KERTESZ, G., 1928, *Wien. med. Wschr.*, **78**, 1136.
- KOLLE, W. & EVERS, E., 1926, *Deuts. med. Wschr.*, **52**, 1075.
- V. KUBINYI, P. & JOHAN, B., 1922, *Zbl. Gynäk.*, **46**, 57.
- LEVADITI, C. & MANOUELIAN, Y., 1905, *C.R. Soc. Biol.*, Paris, **59**, 527.
- LEVADITI, C. & MARIE, A., 1919, *Ann. Inst. Pasteur*, **33**, 741.
- LEVADITI, C. & PETRESCO, G. Z., 1905, *Pr. méd.*, **13**, 617.
- LEVADITI, C. & SAUVAGE, 1906, *C.R. Acad. Sci.*, Paris, **143**, 559.
- LEVADITI, C. & YAMANOUCHI, T., 1908, *C.R. Soc. Biol.*, Paris, **64**, 957.
- MCINTOSH, J. & FILDES, P., 1914, *Brain*, **37**, 141.
- MCNAMARA, W. L., 1925, *Amer. J. Syph.*, **9**, 470.
- MCNEE, J. W., 1922, *Quart. J. Med.*, **15**, 215.
- MARSHALL, C. H., 1925, *J. Amer. Med. Ass.*, **85**, 875.
- MAYER, E., 1921, *Berl. klin. Wschr.*, **58**, 882.
- MATZENAUER, R., 1929, *Wien. klin. Wschr.*, **42**, 2.
- MENNINGER, W. C., 1928, *Amer. J. Syph.*, **12**, 531.
- METCHNIKOFF, E. & ROUX, E., 1903, *Ann. Inst. Pasteur*, **17**, 809; 1904, *ibid.*, **18**, 1,657; 1905, *ibid.*, **19**, 673; 1906¹, *ibid.*, **20**, 785; 1906², cited from D'ARCY POWER & J. KEOGH MURPHY, 1908, *A System of Syphilis*, **2**, 180 and MAISONNEUVE, P., 1906, *Experimentation sur la prophylaxie de la Syphilis*, Thèse de Paris.
- MICHELSON, H. E., 1929, *Arch. Derm. Syph.*, N.Y., **19**, 66.
- MONTPELLIER, J., 1926, *Pr. méd.*, **34**, 451.
- MOORE, J. E. & KEIDEL, A., 1921, *J. Amer. Med. Ass.*, **77**, 1; 1923, *ibid.*, **80**, 818.
- MOORE, J. E. & KEMP, J. E., 1923, *Arch. Intern. Med.*, **32**, 464.
- MORROW, H. & EPSTEIN, N. N., 1928, *Arch. Derm. Syph.*, N.Y., **17**, 309.
- MOTT, F. W., 1914¹, cited from POWER & MURPHY, *A System of Syphilis* (Oxford Med. Pub.), 2nd Edn., **4**, 488; 1914², *ibid.*, **4**, 279.
- NEISSER, A., 1906, *Die experimentelle Syphilis-Forschung nach ihren gegenwärtigen Stände* (Berlin), Springer.
- NEISSER, A., BAERMANN, G. & HALBERSTÄDTER, 1906, *Deuts. med. Wschr.*, **32**, 1, 49, 97.
- NICHOLS, H. J., 1911, *J. Exp. Med.*, **14**, 196; 1914, *ibid.*, **19**, 362.
- NICHOLS, H. J. & HOUGH, W. H., 1913, *J. Amer. Med. Ass.*, **60**, 108.
- NOEGERRATH, C. T. & STAEHELIN, R., 1905, *Münch. med. Wschr.*, **52**, 1481.
- NOGUCHI, H., 1912, *J. Exp. Med.*, **15**, 201; 1913, *Münch. med. Wschr.*, **60**, 737.
- NOGUCHI, H. & MOORE, J. W., 1913, *J. Exp. Med.*, **17**, 232.
- ORR, D. & ROWS, R. G., 1904, *Brain*, **27**, 460.
- PARODI, U., 1907, *Zbl. Bakt.*, Abt. 1, Orig., **44**, 428.
- PEARCE, L. & BROWN, W. H., 1919, *Proc. Soc. Exp. Biol.*, N.Y., **17**, 164; 1922, *J. Exp. Med.*, **35**, 39.
- PLAUT, F. & MULZER, P., 1922, *Münch. med. Wschr.*, **69**, 496.
- RAVAUT, P., 1903, *Bull. Soc. méd. Hôp.*, Paris, **20**, 1026.
- REASONER, M. A., 1916, *J. Amer. Med. Ass.*, **67**, 1799; 1917, *Amer. J. Syph.*, **1**, 478.
- SALLEBY, E. R., 1926, *Amer. J. Syph.*, **10**, 215.
- SCHÖNFELD, W. & KREY, H., 1927, *Münch. med. Wschr.*, **74**, 412.
- SCHUCHT, cited from NEISSER, 1906, *Die experiment. Syph.*, p. 9.
- SÉZARY, A., 1926, *Pr. méd.*, **34**, 4.
- SÉZARY, A. & PAILLARD, H., 1910, *C.R. Soc. Biol.*, Paris, **68**, 295.
- SICARD, J. A., 1925, *Médecine*, Paris, **7**, 141.
- SIMMONDS, M., 1906, *Münch. med. Wschr.*, **53**, 1302.
- DE SOUZA CAMPOS, E., 1923, *Johns Hoph. Hosp. Bull.*, **34**, 253.

- STARRY, A. C., 1924, *Amer. J. Syph.*, **8**, 615.
 THOMPSON, L., 1920¹, *Amer. J. Syph.*, **4**, 323; 1920², *ibid.*, **4**, 50.
 TRICHINESE, J., 1913, *Beitr. Geburtsh. Gynäk.*, **18**, 201.
 TRUFFI, M., 1909, *Zbl. Bakt.*, Abt. I, Orig., **48**, 597; **52**, 555; 1911, *Berl. klin. Wschr.*, **48**, 653.
 UHLENHUTH, P. & MULZER, P., 1910¹, *Arb. Gesundh.Amt.*, Berl., **33**, 183; 1910², *ibid.*, **34**, 222; 1911, *Berl. klin. Wschr.*, **48**, 653; 1913¹, *Beiträge zur experimentellen Pathologie und Therapie der Syphilis mit besonderer Berücksichtigung der Impf-Syphilis der Kaninchen*, Berlin, p. 189, cited by MATZENAUER; 1913², *Berl. klin. Wschr.*, **50**, 769 and 2031; 1913³, *Deuts. med. Wschr.*, **39**, 879.
 WARTHIN, A. S., 1914, *Amer. J. Med. Sci.*, **147**, 667; 1916, *J. Infect. Dis.*, **19**, 138; 1918, *Amer. J. Syph.*, **2**, 425; 1922, *J. Infect. Dis.*, **30**, 569; 1926, *Amer. J. Syph.*, **10**, 1; 1928, *ibid.*, **12**, 301; 1929, *Brit. Med. J.*, ii, 236.
 WARTHIN, A. S. & STARRY, A. C., 1921, *J. Amer. Med. Ass.*, **76**, 234.
 WELCH, A. S., 1928, *Amer. J. Syph.*, **12**, 325.
 WILE, U. J., 1917, *Amer. J. Syph.*, **1**, 84.
 WILE, U. J. & HASLEY, C. K., 1921, *J. Amer. Med. Ass.*, **76**, 8.
 WILE, U. J. & KIRCHNER, A., 1923, *Arch. Derm. Syph.*, N.Y., **8**, 831.

Resistance.

NATURAL RESISTANCE.

There is no evidence of natural resistance by man to invasion by *S. pallida*, though cases have been recorded in which prostitutes who have taken no particular precautions have escaped infection for a number of years (Brandt, 1922).

Non-Reaction at Site of Inoculation.

If the non-development of a primary lesion can be attributed to a partial resistance, this has often been demonstrated in experimental animals. In a small proportion of rabbits, infection is proved only by inoculation of their lymph glands or organs into other animals. Perhaps the most striking example of chancre-less infection is that of mice. Schlossberger (1927) and Kolle and Schlossberger (1928) found that these animals could harbour *S. pallida* for as long as sixteen and a half months after inoculation without showing any active sign of disease, but when portions of their lymph glands, spleen, or brain were engrafted into rabbits these became infected. After a number of passages from mouse to mouse, *S. pallida* was still infective for rabbits. Somewhat similar results have been obtained with rats and guinea-pigs. So far as visible primary lesions are concerned reports of successful inoculation of horses, goats, dogs, pigs and cats have been very scanty and of doubtful reliability, though experiments may show, as in the case of mice, rats and guinea-pigs, that the number of species which are completely resistant is small.

In man, well-proven instances of chancre-less infection have been reported from time to time, and Kolle, as well as Brown and Pearce, Prigge (1929) and others have suggested, by inference from animal experiments, that they may be more numerous than is believed.

Circumstances Affecting the Development of a Primary Lesion.

Size of inoculum. In rabbits, Nichols and Walker (1923), Brown and Pearce (1922), Chesney and Kemp (1925¹) and Wakerlin (1926) found that,

within limits, the incubation period was inversely proportional to the dose of inoculated virus, but when the incubation was prolonged by reduction of the dose, the subsequent course of the disease was not reduced in severity. Probably the dose factor is responsible for the course of events in experiments to determine if *S. pallida* can penetrate intact mucous membrane. Brown and Pearce (1924) inoculated 15 rabbits by instilling into their apparently intact preputial and conjunctival sacs emulsions of *S. pallida* of three strains. Characteristic lesions at sites of inoculation developed in eight ; slight lesions appeared in four ; and in three there was no definite lesion. Nevertheless, subsequent events proved that all had been infected. The incubation period in those animals which showed inoculation lesions was much longer than in rabbits inoculated in the ordinary way, and in several instances scrotal lesions appeared before any at the original sites. The results of experiments, related in more detail below, show that in rabbits the severity of later symptoms is inversely proportional to the extent and severity of the primary.

Trauma. The partial resistance expressed by the non-development of a chancre may be broken down by trauma. Thus Chesney, Turner and Halley (1928) showed that, if wounds are produced in the backs of rabbits, and shortly afterwards when the wounds are granulating, or else healed, the animals are inoculated intravenously, syphilomata usually appear in the wounds, or their scars, with an incubation period much the same as that following an intradermal inoculation.

Influence of drugs and locally applied antiseptics. It seems to be fairly easy to prevent the development of a chancre in a susceptible person or a rabbit by administration of a subtherapeutic dose of a chemotherapeutic agent, or by the application of an antiseptic to the site of inoculation, as in the classical experiment by Metchnikoff and Roux. In man such a case has been reported by Audry (1926) after the administration of stovarsol, and I have seen three cases in which the application of an antiseptic certainly suppressed the primary sore.

In rabbits, Kolle (1924¹) and Kolle and Evers (1926¹) found that, if an injection of a bismuth suspension was given and the animal subsequently inoculated with syphilis, whilst the bismuth depot remained, no chancre followed. If, after inoculation, the bismuth depot was excised, though this was two to three months later, a chancre followed the excision in about eight weeks. The bismuth prevented the development of the chancre, but not the spread of virus into the tissues, for before removal of the bismuth depot the glands were shown to be infective for other rabbits.

ACQUIRED RESISTANCE.

The question of acquisition of resistance to syphilitic infection has given rise to considerable controversy. It is agreed that the tissues are unable without therapeutic assistance to rid themselves of an original infection, and no undoubted case of spontaneous cure has been recorded in man. In rabbits, as was shown by Brown and Pearce (1921¹) and

generally accepted, after subsidence of generalized lesions, the lymph glands remain infective for an indefinite time. Eberson and Engman (1921) showed similarly the persistence of virus in the lymph glands of human subjects with latent syphilis.

Although the tissues are unable to rid themselves completely of *S. pallida*, the long periods of latency and the great ages to which human syphilitics can live are evidence that they can resist its attack. Whether this resistance is due merely to a tolerance of the parasite ('Anergie'), to a neutralization of its toxins, or to an active destruction of the germ, are questions on which opinions are divided.

Conditions Affecting the Acquisition of Resistance.

Development and extent of the primary lesion. Brown and Pearce found that it was often possible to modify the course of the disease in rabbits by experimental intervention, and showed that the degree of interference with the interaction between the virus and the tissues is an important factor determining the severity of subsequent lesions. In one series of experiments (1920¹, 1921²) they showed that animals inoculated in one testicle were more prone to develop generalized lesions than those inoculated in two testicles. If the inoculated testicles were removed, the advantage of bilateral inoculation was lost. If in an animal which had been bilaterally inoculated one testicle only was removed, there was less likelihood of generalized lesions than if both were removed.

The effect of administration of a subcurative dose of a therapeutic agent was studied in 24 rabbits, the drug being arsenophenylglycyl-m-aminophenol, which acts more on lesions than on spirochaetes. Half the rabbits were bilaterally inoculated and half unilaterally, and 14 days later 6 of each group were injected with 5 mgm. of the drug per kgm. of body-weight. In the bilateral series 5 of the 6, and in the unilateral series all, developed generalized lesions, while in the untreated controls only 1 of the 6 bilateral and 3 of 6 unilateral did so.

In an analysis of the different types of generalized lesions which followed various degrees of interference with the development of the primary granuloma, Brown and Pearce (1921²) suggested that certain groups of tissue tend to become involved in a given order, bones, skin and mucous membranes, then eyes; that by early suppression of the primary lesion the protection may not reach bones, which are then likely to become diseased, with resulting protection passed on to skin and mucous membranes, and perhaps further to eyes. Later intervention, on the other hand, may find bones already protected, but not the skin and mucous membranes and eyes, the recurrence then occurring in the former. Interference at a still later stage in the development of the primary lesions may result in bones and skin escaping the recurrence but not eyes. Brown and Pearce suggest that, if this obtains in man, it may explain the development of such conditions as neurosyphilis. Most clinicians would probably agree that there is much in this suggestion,

which has been made also by Nichols (1911) and numerous other workers since. Two features of a large proportion of syphilitic neuro-recurrences are prominent, namely, triviality of the primary sore, or an abrupt interference with the development of early lesions by a subcurative treatment with arsenobenzene compounds.

The following factors affecting resistance may be mentioned :

Light. The resistance of inoculated rabbits varies directly with the amount of light to which the animals are exposed (Brown and Pearce, 1927).

Vaccinia. Rabbits inoculated in the testicle with syphilis and at the same time in the skin with vaccinia develop severe syphilis, but rabbits immunized to vaccinia, as well as those inoculated intratesticularly with both vaccinia and syphilis, develop a milder disease (Pearce, 1928).

The endocrine system. This is affected in syphilitic rabbits, and Brown and Pearce (1923) noted changes in the thyroid, parathyroids, suprarenals, the hypophysis, and the thymus.

Pregnancy. The effect of pregnancy in modifying syphilis in women is well known ; thus, a woman may give birth to a syphilitic child and suckle it without showing any obvious sign of syphilis. In women who become pregnant after infection abnormalities of the cerebrospinal fluid are less frequent than in women who do not become pregnant, and clinical manifestations tend to be suppressed in the former (Moore, 1923).

Brown and Pearce (1920^a) inoculated 8 pregnant rabbits with syphilis. Only 4 showed any clinical sign of infection, and in 3 of these there was only a very slight and transient infiltration at the sites of inoculation. Two of these, however, showed very marked constitutional disturbance, and of 15 young born of these 2, only 1 survived beyond the first week. The fourth rabbit had been inoculated in the middle of pregnancy and showed a lesion only at the end.

Resistance to Superinfection and to Reinfection in Man.

It has long been known that in man from a date shortly after the appearance of a primary lesion reinoculation usually fails to provoke a second chancre. John Hunter (1810) inoculated a patient with his own virus in the secondary stage, and the result was negative. Various writers have remarked on the resistance of syphilitic patients to second inoculations, a resistance which, so far as the development of a second chancre is concerned, grows with the age of the infection, and, with few exceptions, becomes practically absolute by the end of 14 days. Thus Queyrat (1906), in 19 auto-inoculations of patients with primary syphilis, obtained 10 positive results, but none was in a case with a chancre older than 11 days. Levaditi, Laroche and Yamanouchi (1908) observed that the establishment of the refractory state practically corresponded with the change of the Wassermann reaction to positive. Finger and Landsteiner (1906), by introducing large amounts of virus into the skin, were able to produce lesions in stages later than the primary, but these

resembled the lesions which the patient exhibited at the time of the second inoculation. Control experiments with dead virus gave negative results. These experiments go to show that the changing character of the reaction of the tissues to the virus depends on changes in the tissues rather than in the spirochæte. Hashimoto (1926) inoculated 20 syphilitics with fresh virus, and new primary lesions resulted in 6 cases. He concluded that superinfection is possible, and, contrary to the common belief, that the new lesions do not correspond in character to the stage of the original infection. Lacapère (quoted by Bernard, 1926) remarks that among the Arabs, chancres are frequent in persons presenting the stigmata of hereditary syphilis, and he considers that binary syphilis is common in this race. He notes that syphilitic men, after contact with women the subjects of vulvar lesions, frequently develop chancriform lesions, which he regarded as the result of superinfection. Lacapère had not, however, seen a second infection in an hereditary syphilitic with a positive serum reaction. Other workers have seen what they described as new chancres on syphilitics with old infections, but the difficulty seems to be to decide if these new lesions were chancres or chancriform gummata. I have, however, seen a chancre on an adult with classical stigmata of congenital syphilis with negative serum reactions. *S. pallida* was found in the exudate from the sore, and treatment was withheld until secondary symptoms began to appear. It seems probable, therefore, that, after the activity of the original infection has died down to such a degree that the serum reactions are negative, the skin may again react to a new inoculation in the same manner as to a primary inoculation. Bernard (1926), reviewing the mass of conflicting statements on this subject, expresses the opinion that superinfection of a person still harbouring *S. pallida* is possible long after the primary stage.

The refractory state of the skin so far as the production of a second chancre is concerned, which develops in man as a result of the first infection, appears to extend to all strains of *S. pallida*, unlike the case in lower animals, as will be shown later. There is also some evidence to show that, to some extent, it applies in man also to *S. pertenuis*, since Jahnel and Lange (1925) failed to produce a primary yaw in any of a number of general paretics by inoculating them with *S. pertenuis*, though a normal person inoculated at the same time was infected. In later experiments, however, with another strain of *S. pertenuis*, Jahnel and Lange (1927) succeeded in infecting a general parietic.

Chancreless superinfection. So far the discussion has been on the possibility of superinfection based on the evidence of a new skin lesion, but although no external lesion may follow reinoculation of a syphilitic man, it does not follow that the reinoculated virus has not spread into the tissues. Considerable evidence that this occurs in rabbits will be discussed later; in man, proof of its happening is naturally difficult to obtain. Prigge and Ruthowski (1929), however, demonstrated what they believed to be a symptomless superinfection in a general parietic.

So far, therefore, as syphilis in man is concerned, the evidence is that the tissues soon develop a non-reactivity to new virus introduced from without, but whether this is due to mere 'anergy' or manifests a true immunity cannot be stated.

Development of allergy. The long periods of latency of the disease suggest also the development of a non-reactivity to the virus *in situ*, but this non-reactivity is liable to be interrupted by a reaction which is greater in relation to the demonstrable virus than is found in earlier stages. Thus, in the primary stage one finds a moderate reaction to large numbers of spirochætes, but in the tertiary, while spirochætes are scanty, the tissue reaction is great, a state usually attributed to a change in the tissues (allergy), not in the parasite, because infections produced by inoculation with gummatous material commence with lesions which are primary in type. Other evidence that the governing factor in the reaction is the state of the tissues is that inoculations from malignant syphilis produce normal syphilitic reactions.

In this connection, it is interesting that late recurrences may be provoked by trauma, and often spread far beyond the site of the injury. I have observed a nodular cutaneous syphilide which started at the site of a prick on a hypothenar eminence and spread gradually across the palm to the other side. Conceivably, a pre-existing balance between the tissues and the virus is broken down by the trauma, with a temporary increase in virulence of the local parasites.

Superinfection and Reinfection in Animals.

In rabbits superinfection with resulting new chancre has been demonstrated by various workers, e.g. Brown and Pearce (1921³) and Frei (1923). Moreover, successful reinoculation does not prove eradication of the original infection. These superinfections of animals with production of chancres were carried out in the early days of the original infection, and it will be shown that the older the infection in rabbits the less the chance of provoking a new chancre by reinoculation. There is also evidence that, just as the primary chancre influences the subsequent development of lesions in other parts of the body, as shown by Brown and Pearce, so this factor is important in preventing a chancre resulting from reinoculation. Manteufel and Richter (1926) showed that after intravenous inoculation rabbits may become infected, as proved by inoculation of their lymph glands into other rabbits, but reinoculation of six such rabbits intradermally or intratesticularly resulted in five developing primary chancres. Also Prigge and Rothermundt (1927) have shown that rabbits which develop no chancre after inoculation into the testicle or the skin, but are nevertheless infected, may develop chancres after a second inoculation, though the reinoculation may be with homologous virus and more than ninety days after the first. The absence of any chancre immunity (as Kolle terms it) in cases where no chancre has resulted from the first inoculation may explain Neisser's view that monkeys

could only rarely be infected by intravenous, intraperitoneal, or subcutaneous inoculation. The non-appearance of any local lesion after inoculation by such routes and the subsequent production of chancres by inoculation into the skin were interpreted as failure of the first inoculation, but cross-inoculation of lymph glands into other animals might easily have proved them to be suffering from a latent infection at the time of the second inoculation.

Whatever the underlying cause of the non-development of a new chancre after reinoculation, it is agreed that it results from interaction between spirochaetes and skin, and this for a minimum period.

The time factor was demonstrated more precisely by Kolle (1922, 1924²), who showed that, if a rabbit with an infection of less than 45 days' duration is treated with neosalvarsan in sufficient doses and is then reinoculated into the same site, even with the same strain of virus, a new chancre usually results. If treatment is delayed until the infection is between 45 and 90 days old, the success of a reinoculation so far as a new chancre is concerned is variable, and, if treatment is not instituted until after the ninetieth day, reinoculation practically never provokes a new chancre. Kolle, following the Neisser dictum, inferred from this that treatment of a syphilitic rabbit after the ninetieth day practically never eradicates the disease. Chesney (1927) collected the results of a number of workers' experiments on the same lines, which practically confirmed those of Kolle. This applies only to homologous strains; when heterologous strains are employed for the reinoculation, chancres follow more frequently, as shown by numerous workers.

Effect of trauma on resistance to reinfection. The resistance against reinfection with homologous strains can be broken down by injury, as demonstrated by Chesney and Kemp (1926), who treated 16 rabbits with '606' from 159 to 201 days after inoculation into the testicle, and 89 days later reinoculated with homologous virus on granulating wounds of the back. Only one animal developed a chancre, but seven others were proved by lymph-node transfer to have been reinfected.

The inference as to eradication of the original infection which can be drawn from success or failure of reinoculation is a matter of dispute. As mentioned, Kolle, following the dictum of Neisser (1911), interpreted failure to reinfect as meaning persistence of the original infection. Others, though substantially confirming Kolle's results, dispute his interpretation. Chesney and Kemp (1924, 1925²) after treatment of their animals showed them to be sterile by negative results of transfer of their lymph-nodes to other animals and consequently believed that resistance to reinfection is due to an immunity which commences with chancre immunity and develops into complete resistance. The weight of evidence seems to support Chesney and Kemp's contention. Although they were unable to demonstrate persistence of the original infection in their refractory animals after treatment, the fact that in 50 per cent. of cases they were able to reinfect on producing granulating wounds seems to show that

tissue resistance rather than presence of original spirochætes is the dominating factor. Breinl and Wagner (1929), Voegtlin and Dyer (1925) and Uhlenhuth and Grossmann (1928) hold the same views.

Breinl and Wagner pointed out that to accept the theory that susceptibility to reinfection occurs contemporaneously with the death of the last spirochæte is to assume that the tissues had not been changed by the presence of the spirochæte. As a result of experiments to determine the fate of *S. pallida* after reinoculation into animals which had been cured after their infections had lasted varying periods, they found that all those treated from the eleventh to the twenty-sixth day after the primary inoculation were susceptible to reinfection, as well as those treated from the forty-sixth to the fiftieth day, though in the latter group there was a retardation of the spread of the reinfection. In a third group, treated from the ninety-third to the one hundred and thirtieth day after first inoculation, three animals were susceptible, and two were not susceptible, to reinfection. In a fourth group, treated from 274 to 412 days after inoculation, only one seems to have been infected by reinoculation. The development of resistance was considerably slower in some animals than in others; also, as Chesney and Kemp have pointed out, the usual first sign of commencing resistance was a chancre immunity.

Uhlenhuth and Grossmann's experiments also support Chesney and Kemp's contention that failure to infect by reinoculation is due not to persistence of the original infection, but to the slow development of a resistance which lasts for a considerable, at present unknown, period after eradication of the original infection.

In spite of this, Kolle and colleagues remain unconvinced, holding that, although animals treated after the ninetieth day of infection do not, as a rule, respond to reinoculation with a chancre, they are, nevertheless, infected, the virus spreading through the body without causing symptoms. They say, in fact, that the so-called immunity is only a chancre immunity. Kolle and Prigge (1929) said that Chesney must have overlooked the possibility of symptomless reinfection. This statement is difficult to understand, for Chesney and Kemp tested their reinoculated animals by gland transfer to other rabbits. Kolle and Prigge reinvestigated the question. They treated a number of syphilitic rabbits with neosalvarsan 3 to 8 months after first infection, and 6 months later reinoculated some with homologous and some with heterologous virus. Six to eight months after the reinoculation the animals' glands and pieces of testicle were transferred to other rabbits. In the case of the controls which had been treated but not reinoculated the eventual gland transfer proved negative. In the others, of 27 rabbits reinoculated with homologous virus, 6 showed lesions and 18 of the remainder were proved by gland transfer to have been reinfected. In the series of 26 rabbits reinoculated with heterologous virus, 14 developed lesions and 8 of the remaining 12 had infective glands. To explain the difference in their results, Kolle and Prigge suggest that Chesney and Kemp reinoculated too soon after the treatment,

when remnants of '606' would still be present and prevent the reinoculated virus from infecting; also that they cross-inoculated too early after the reinoculation. Reference to Chesney and Kemp's later paper shows that from end of treatment to reinoculation, 69 days, and from first reinoculation to second, 39 days elapsed. It is difficult to believe that a course of treatment with '606' would prevent infection from an inoculation 69 days later. As regards the contention that the cross-inoculation to test the success or otherwise of reinoculation was carried out too soon, Kolle and Evers (1926²) showed that after a primary inoculation the virus was in the popliteal glands of a rabbit in half an hour, and Brown and Pearce (1920³) had previously shown that it was in the inguinal glands in less than 48 hours after inoculation. It seems difficult to escape the conclusion that the reason for Chesney and Kemp's failure to find evidence of gland infection after the first and second reinoculations was that something other than remnants of '606' given 69 days prior to the first reinoculation prevented the multiplication of the spirochætes.

As already mentioned, Chesney and Kemp showed that local injury can break down resistance to reinoculation, and possibly this factor may explain Kolle and Prigge's success. Again, Brown and Pearce have demonstrated the effect of intensity of the reaction at the site of inoculation on the resistance of other parts of the body. If Chesney and Kemp's animals had larger primary lesions than had those of Kolle and Prigge, they might, conceivably, have developed a stronger resistance.

Kolle and Prigge, holding apparently still to the Neisser dictum that immunity, even chancre immunity, means persistence of the original infection, suggest that Chesney, Uhlenhuth and Grossmann and others, who contend that negative cross-inoculation from animals treated with '606' late in the disease indicates sterilization, appear to have lost sight of the possibility of the virus being still present in the bone-marrow. They quote the work of Worms (1926) who found that rabbits treated late in the disease with quite large doses of '606' were not always sterilized. Worms also found that failure of the first cross-inoculation to produce lesions in the second series of rabbits did not necessarily prove that the first series had been cured, as further cross-inoculations from the symptomless second series to a third series could result in lesions.

Prigge (1928) showed that in 3 out of 4 rabbits which had been treated with a subcurative dose of '606' only 15 days after inoculation and been found on reinoculation later to be chancre-immune, cross-inoculation with lymph glands had proved negative. The period to first treatment was too short for the development of a chancre-immunity, and only persistence of the original infection would explain it, yet the glands failed to convey infection.

In human syphilis reinfection is recognized only when a second chancre develops. It is certainly interesting that, as Halley and Wassermann (1928) demonstrated in an analysis of 237 well-established cases which they had collected from the literature and from their own clinic, the vast

majority of reinfections which have been published have been in patients treated in the first three months of the disease. It is possible that in human beings, as in rabbits, reinfections occur in persons treated later, but the reinfection does not become apparent because the patients have become chancre-immune. Such would explain, as Chesney and Kemp (1926) have suggested, some of those cases in which, after very thorough treatment followed by negative serum reactions for many months or years, the serum reactions have suddenly become positive. Prigge (1927) has made a similar suggestion.

Influence of site of primary lesion. The experimental work so far discussed has shown that an essential condition of chancre-immunity is the previous development of a primary lesion in the skin or testicle, since it has been shown that animals which do not develop a chancre on first inoculation and those which have been inoculated intravenously can respond with a chancre to a second inoculation, though still suffering from a latent infection. It is interesting in connection with the well-known intractability of interstitial keratitis that when the primary inoculation of a rabbit is into the anterior chamber of the eye, with resulting keratitis, an inoculation into the testicle or scrotum after healing of the keratitis usually results in a chancre (Uhlenhuth and Mulzer, 1913). On the other hand, when the primary inoculation has been into the testicle and the second into the anterior chamber the results have not been so consistent. Thus, Uhlenhuth and Mulzer obtained only one keratitis in 11 rabbits reinoculated from 59 to 170 days after the first. Tomaszewski (1910), on the other hand, by corneal reinoculation produced keratitis in 16 of 21 rabbits inoculated into the testicle. Frei (1923), by reinoculation into the eye after first inoculation into the scrotum or testicle, produced 4 keratitis in 25 rabbits. The majority of workers seem, in fact, to have been more successful in producing a second chancre when the primary lesion was in the eye and the reinoculation was into the scrotum or testicle than when the order was reversed. According to Uhlenhuth and Weidanz (1908), a primary keratitis of one eye does not usually protect the other cornea against superinfection.

SUMMARY.

The available evidence appears to justify the conclusions that :

(a) There is little or no evidence of any natural resistance in man to infection with *S. pallida*.

(b) Monkeys, rabbits, guinea-pigs and mice have been proved to be susceptible, though infection may not be followed by the development of a primary lesion.

(c) Prevention of the primary lesion is comparatively easy, and its absence is the rule in some animals, e.g. mice.

(d) The question of the development of true immunity as a result of infection is still under discussion, but it is admitted that :

(i) If the first inoculation does not produce a primary lesion of the skin or testicle, reinoculation may easily result in a chancre.

(ii) The production of a second chancre by reinoculation, even after apparent sterilization, becomes increasingly difficult the longer the disease has lasted before sterilization is effected.

(iii) Reinoculation with heterologous virus is more likely to result in a second chancre than is reinoculation with the virus used for the first inoculation. Homologous virus is more likely to infect if inoculated into a granulating wound.

(e) A proportion of rabbits in which a primary lesion of the skin or testicle followed the first inoculation, and the infection has persisted for a considerable time (three or more months) before sterilization, not only develop no chancre on reinoculation, but show no evidence of reinfection in their lymph glands. This, in contrast with the high proportion of infective lymph glands in animals suffering from original infections, points strongly to the gradual development in syphilitic rabbits of a definite resistance to reinfection, even though this may not be absolute.

(f) Granting the development of some degree of immunity in syphilitic rabbits and other animals, there is no evidence that this immunity is ever able to rid the animal of its original infection.

Antibodies in the Serum.

AGGLUTININS.

Levaditi (1905) observed an agglutination of *S. pallida* in specimens made from the contents of bullæ in syphilitic pemphigus, and Hoffmann (1906) a slowing of their movements in syphilitic serum, but the effect does not appear to be constant or of diagnostic value in respect of the serum of syphilitic patients and infecting virus. Scharnke and Ruete (1921) reported a strong immobilizing effect of spinal fluid from a secondary syphilitic and also particularly from general paretics. They found that the fluid from paretics was more immobilizing than their serum.

Kissmeyer (1915, 1917), besides demonstrating agglutination of cultural *S. pallida* by high dilutions of serum of immunized rabbits, found that 40 to 60 per cent. of the sera from 59 cases of primary and tertiary syphilis agglutinated cultural *S. pallida* in dilutions of 1/100, normal sera agglutinating in a dilution of 1/50. Mano (1928) tested 100 sera by agglutination of cultural *S. pallida* (an old strain) and by Wassermann methods. Both were positive in 48 cases and both negative in 7. The Wassermann reaction was negative, but agglutination positive in 38, and the reverse of this occurred in 7. Kolmer (1913), Zinsser and Hopkins (1915), Wilkes-Weiss, Richter and Kolmer (1926) and Dold and Worms (1928) all obtained agglutination of cultural *S. pallida* with considerable dilutions of immune sera of rabbits and horses inoculated with cultural *S. pallida*; the serum of untreated animals agglutinated not at all or only in low dilution. Zinsser, Hopkins and McBurney (1916) did not

find, however, any agglutinating effect of their immunized rabbits' sera on *S. pallida* direct from human lesions, and Wilkes-Weiss, Richter and Kolmer, who used six cultural strains in their immunization work, found no evidence of strain specificity. Kast and Kolmer (1929) have, however, recently expressed strong doubts of the identity of the cultural spirochætes used in these experiments with the organism of syphilis. Cohn (1929¹) found that the serum of rabbits immunized by intravenous injections of cultural *S. pallida* actively agglutinated these organisms. On the other hand, the serum of syphilitic and healthy persons did not agglutinate cultural spirochætes except after a series of injections of the same organisms.

SPIROCHÆTICIDINS.

Eberson (1921) produced evidence that the serum of syphilitic rabbits and men in certain stages when mixed with virulent *S. pallida* prevented infection, but his work does not appear to have been repeated.

EFFECTS OF 'ANTISERA' IN PROPHYLAXIS AND TREATMENT.

Jaureguy and Lancelotti (1924) stated that they had reproduced syphilis in South American llamas and had cured them, and also human syphilitics, with serum of llamas immunized by pure cultures. None of numerous other workers who have repeated these experiments has obtained any such result. Also, Quéry's serum, which is obtained from monkeys after inoculation with filtrate of broth cultures of *S. pallida*, has been found to have no protective value. Bruck (1928) and Dold and Worms (1928) found no prophylactic value in serum of horses immunized with cultures of *S. pallida*, and Reiter (1928) failed to protect rabbits by intravenous injections of living cultures against subsequent inoculation with testicle virus.

SPIROCHÆTOLYSINS.

Evidence of a solvent effect of the serum of syphilitic men or animals on virus of infection is lacking, but animals immunized with cultural *S. pallida* appear to develop spirochætolytic properties. Thus, Nakano reported that the serum of rabbits which had been treated with cultures of *S. pallida* disintegrated this organism in the peritoneal cavity of normal guinea-pigs, while normal rabbit's serum had no such action; it did not act *in vitro*. Kroó, Schulze and Zander (1929) reported that the serum of rabbits which had been immunized with cultures of *S. pallida*, when used in the preparation of culture medium, prevented the growth of these organisms and when added to *S. pallida* growing in culture gradually caused their disappearance; spirochætes sown on medium made with immune serum in 1 to 2 hours became more or less motionless, swollen and agglutinated. Kroó and Schulze (1929) reported that the spirochætolytic effect of two immune sera obtained by inoculation of rabbits with two strains of cultural *S. pallida* was strain specific. Cohn (1929²), working with the same strains, reported similar results with sera of men

and of rabbits after immunization. Bergel (1928) mentioned that under dark-ground illumination he had observed a spirochætolytic effect of peritoneal exudates rich in lymphocytes.

COMPLEMENT FIXATION AND PRECIPITATION.

Wassermann, Neisser and Bruck published in 1906 the details of a complement-fixation reaction in which the antigen was a watery extract of syphilitic foetus's liver. The authors believed that the active agent in their extract was *S. pallida* present in the liver, in other words that the reaction was a true Bordet-Gengou phenomenon, demonstrating antibody to *S. pallida*. Considerable doubt was thrown on this theory when Marie and Levaditi (1907) found that extracts of normal organs could be substituted for extract of syphilitic foetus's liver, a discovery which was quickly confirmed by many other workers (some of whom showed that such artificial products as lecithin and cholesterol, oleate of soda and glycocholate of soda would also act as 'antigen'), and by far the most commonly used 'antigen' to-day is an alcoholic extract of heart-muscle, usually with the addition of cholesterol.

Soon after the complement-fixing properties of syphilitic sera were discovered, it was found that, in certain circumstances, they gave rise to precipitation phenomena, and numbers of ingenious tests were proposed, but none became established until Sachs and Georgi (1918) published their flocculation test. The Sachs-Georgi and other tests based on the same principle will be discussed later (p. 251). Here it suffices to say that syphilitic sera fix complement in the presence of heart-extracts and usually also show precipitation phenomena with the same extracts, and it may be taken for granted that a serum which gives a positive Wassermann reaction is positive also to a good flocculation test.

After the discovery of the Wassermann test, considerable discussion followed on the nature of the complement-binding property of syphilitic sera, some holding that it is due to an unstable albumin, or to a mixture of lipoids and lipoproteins which interacts with lipoids in the extract to bind complement, others that it is due to a change in the colloidal condition of the serum, and still others ascribe it to the presence in the serum of antibody to lipoid. For a survey of views on the physico-chemical explanation of the Wassermann test the reader is referred to the critical review by Eastwood (1920).

According to Eagle (1930), who gives convincing details of his experiments, the basic principle of the Wassermann is identical with that of the flocculation reactions. An alcoholic extract of an organ suitable for either reaction when diluted with water is converted from a molecularly dispersed solution to a colloidal one in which the particles (for the most part invisible under dark-ground illumination) are hydrophilic. When a non-syphilitic serum is added to such a sol it forms a protective film round the lipoid particles, shifting the cataphoretic potential and the isoelectric point towards those of serum protein. This film can be removed by washing

and does not prevent the subsequent combination of the underlying lipid with the specific component in syphilitic serum.

When, on the other hand, a syphilitic serum is added, the protein film is formed as before, but, in addition, the lipid forms an irreversible combination with the specific component (reagin) contained in the globulin of the serum, and the combination is hydrophobic. The critical potential is raised from its original value to that of particles of denatured globulin, or of any antigen-antibody complex, and relatively small quantities of electrolytes suffice to depress the stabilizing potential below this level, with resultant aggregation of the particles. The precipitate cannot be freed of its globulin by washing and has a strong affinity for complement. When heated to 100° C. the globulin, and hence the complementophile property is destroyed, and the antigen again becomes active, able to react with more syphilitic serum. Complement fixation and flocculation are merely two methods of demonstrating the presence of the same reagin-globulin complex. The cohesion and flocculation of the protein-coated particles takes place most rapidly at $\text{pH } 3.5 \pm 0.5$, is enormously increased by shaking, by decrease in total volume, by bivalent cations, or by an increase in temperature up to 56° C. Union with mid-piece of complement, on the other hand, takes place at pH 5.8 to 6.2 and at a NaCl concentration of 0.07 to 0.25 N, is only slightly affected by volume and shaking, is completely suppressed by relatively slight concentrations of bivalent cations, and, because of the thermo-lability of the complement, must be carried out at a temperature below 40° C.

The role of cholesterol, itself inert as an antigen, is to form a surface on which the lipoidal film is spread, thus making the colloidal dispersion much coarser. The colloidal suspension has the properties, in respect of stability, of an organ lipid suspension and not those of cholesterol or of any chemical combination of cholesterol with lipid. In contact with a syphilitic serum the same reagin-lipoid complex is formed as when the lipoidal particles have no core of cholesterol, but the properties of both aggregation and of avidity for complement are increased. Eagle regards cholesterol as an unsatisfactory sensitizer from several points of view on account of its small solubility in alcohol. He says: 'Its sensitizing action increases indefinitely with its concentration. If it were sufficiently soluble, even 3 per cent. could be used to advantage, increasing the sensitivity of $1\frac{1}{2}$ per cent. antigen for complement fixation some 200 to 400 per cent., instead of about 50 per cent., as does 0.2 per cent. cholesterol'. Accordingly he has sought for a water-insoluble substance, very soluble in alcohol, with so high an interfacial tension against water that, as in the case of cholesterol, microscopic particles would adsorb antigen when the alcoholic solution of the two is dropped into water. He has found a group of substances fulfilling these expectations and making possible 'an antigen which is from two to ten times as efficient in the Wassermann test as any now available'.

A question which is greatly discussed is whether or not it is possible to demonstrate antibodies to *S. pallida* by complement fixation and

precipitation. Craig and Nichols (1912) tested a number of human sera for complement fixation, using as antigens alcoholic extracts of cultural *S. pallida*, *S. pertenuis* and *S. microdentium*, and also of syphilitic foetal liver. Normal sera (16) and sera from diseases other than syphilis (37) gave negative reactions with all four antigens. Of 51 syphilitic sera, 47 gave positive reactions and 4 negative ones (these were treated cases) with the liver antigen, but all the spirochætal antigens gave several negatives among those positive with the liver antigen. The sera of rabbits—4 normal, 4 syphilitic, 2 frambœsial—were also tested. All were negative to all four antigens, except one syphilitic serum, which was positive to the liver antigen. Craig and Nichols therefore conclude that spirochætal antigens cannot be relied upon.

The question of antibody has been revived in recent years by the reports of Sachs, Klopstock and Weil (1925) and of Sachs and Klopstock (1926) that when rabbits are injected with alcoholic extract of heart mixed with foreign (pig's) serum, the rabbit's serum behaves, in complement fixation with cholesterolized heart-extract, like syphilitic serum. They inferred from this that syphilitic serum contains antilipoid. F. Klopstock (1926) contended that the lipoid was spirochætal. He found that injections of alcoholic extract of *S. pallida* culture evoked Wassermann changes and maintained that the Wassermann reaction in man depends on generalization of the virus, not on tissue changes. By extracting cultural *S. pallida*, he obtained an antigen which acted in complement fixation with syphilitic serum like alcoholic extract of organs. Alcoholic extracts of cocci, *B. coli* or *B. tuberculosis* did not act, and an extract of the culture medium for *S. pallida* gave reactions with syphilitic sera only in concentrations of 1 to 3, as compared with 1 to 5 and 1 to 7 with spirochætal extract. Rabbits injected with alcoholic extract of *S. pallida* in a few days developed complement-fixing properties which were strongest with spirochætal extract, next with Wassermann organ-extract and least with extract of culture medium. Animals injected with extract of culture medium (which contained horse-serum) gave only feeble reactions with spirochætal and culture-medium extracts, but stronger with Wassermann extract. F. Klopstock, therefore, attributed the reaction to the protein-lipoid content of the spirochætes and not to horse-serum of the culture medium adherent to the spirochætes. Thus, his view was that the complement-fixing properties of syphilitic serum are primarily anti-spirochætal, with a group affinity for organ lipoids. Sachs and A. Klopstock (1927) admitted that F. Klopstock seemed to have produced antibody to spirochætal lipoid which acted in tests with this and also to a slighter extent (possibly by a group action) with ordinary extract of organs as used in the Wassermann test. They maintained that the Wassermann reaction depends upon antibody to lipoid, but whether organ or spirochætal lipoid they left an open question. In a later paper F. Klopstock (1928) advanced further arguments in support of his view of the antispirochætal properties of syphilitic serum. He said that the

Wassermann reaction appears in man when tissue destruction cannot be great enough to be the cause, but the multitude of *S. pallida* can, that it appears early in monkeys after injection of *S. pallida*, and that it does not appear in other diseases that are accompanied by great destruction of tissues. He found alcoholic extracts of cultural *S. pallida* washed free from horse-serum as good for the Wassermann reaction as were the customary extracts of organs, and he referred to similar results by Hecht and by Blumenthal and Jaffe. He explained the failure of Kroó and Schulze (see below) to evoke Wassermann reactions by injection of spirochætal extract on the grounds that another strain of *S. pallida* grown on another medium was used and injected intramuscularly, whereas he had employed the intravenous route. Hoeltzer and Popoff (1928) substantially confirmed F. Klopstock's results. They found also that human sera which gave positive Wassermann reactions and some with negative reactions were positive with their *pallida* extracts. Whether this was due to non-specificity of the *pallida* antigen or to its finding an antibody they were not prepared to say. Gaëhtgens (1929), after referring to divergent results obtained with aqueous extracts and suspensions of *S. pallida*, reported success with an antigen composed of cultural *S. pallida* washed with saline and suspended in 0·5 per cent. phenol, the results agreeing with the Wassermann test performed with ordinary cholesterolized heart-extract. Cultural *S. pallida* suspended in phenol but not previously washed failed because the adherent culture medium absorbed the phenol. Similar extract of *S. dentium* also gave positive reactions, just as F. Klopstock had found with alcoholic extracts of *S. pallida* and of *S. dentium*. Phenolized extracts of *B. coli* of *B. typhosus* and of yeast often afforded positive reactions with syphilitic sera, though not so strongly as did extract of *S. pallida*, but, unlike this, reacted with non-syphilitic sera. Rabbits and guinea-pigs inoculated with the phenolized *pallida* antigen developed strong complement-fixing properties with the homologous antigen and weaker reactions with ordinary Wassermann extracts, and Gaëhtgens concluded that it was probable that the two reactions—the Wassermann and the anti-*pallida*—were not the same, the Wassermann being a reaction with lipoids in the *pallida* extract and the specific reaction being due to true antibody. Blumenthal (1929) doubted the inferences deduced by Sachs, Klopstock and Weil from their experiments. He referred to observations by numerous workers which go to show that normal rabbits' sera may become Wassermann-positive without any intervention, or after injection of a variety of substances, e.g. kidney and liver extracts. After intraperitoneal injections of lipoids from ox or rabbit's liver, yeast, and mixtures of these into rabbits, some sera quickly became Wassermann-positive, others remained negative for a time after the injections had ceased and then suddenly became positive. As metabolism appeared to be concerned, he tested fasting animals and found that some became more strongly positive, and he thought that the cause lay in the increase of amino-acids in the blood, as suggested by

Racchiusa (1921-2) and others. For these reasons Blumenthal thought that the results of Sachs, Klopstock and Weil were due to excess of amino-acids produced in the rabbits' blood by the intravenous injection of lipid and pig's serum and the great lability of rabbits' tissues. In support of this explanation, he mentioned that so far other workers had failed to repeat the results of Sachs, Klopstock and Weil with other animals, e.g. Henning (1928) with guinea-pigs; Heronimus and Awrech (1927) with rats; Frei and Grünmandel (1927) and Fortig (1927) with human beings. He pointed out that Kroó and Schulze (1928), by injecting human beings with washed cultures of *S. pallida*, had obtained complement-fixing bodies, but these acted only with *pallida* antigen, not with heart-extracts.

In order to elucidate the problem, Blumenthal decided to try intrathecal injections as likely to afford results less disturbed by complicating factors. Blumenthal considered that the suggestion of Neufeld and Szye (1928) that antibodies wandered into the cerebrospinal fluid from the blood-stream might be ruled out. After a series of tests had shown that the cerebrospinal fluid of normal rabbits does not contain bodies fixing complement with Wassermann extracts, he gave rabbits a number of intrathecal injections of rabbits' liver lipoids mixed with pig's serum in the same concentrations as employed by Sachs and colleagues. The results of 5 to 15 injections were quite negative, and the same followed injections of rabbits' and of horse's kidney lipoids, each in conjunction with pig's serum. On the other hand, daily intrathecal injections of washed cultural *S. pallida* resulted in the appearance of complement fixation which was strongest with *pallida* antigen and with extract of syphilitic liver and weaker with heart-extracts. A further series of experiments in which a mixture of alcoholic extract of cultural *S. pallida* (spirochætal lipid) and pig-serum was injected gave completely negative results, as did also injections of spirochætal lipid without serum. Thus, only intrathecal injection of aqueous suspension of *S. pallida* resulted in the appearance of any complement-fixing property in the spinal fluid, and this, though strongest with *pallida* antigen, was also manifested with Wassermann antigen. In the production of the Wassermann reaction in human syphilis, Blumenthal therefore concluded that an essential part is played by spirochætal substance as originally conceived by Wassermann and believed by F. Klopstock. At the same time, he thought that the Wassermann reaction depends on a non-specific as well as a specific factor.

Kroó and Schulze (1929) followed up the results referred to by Blumenthal (in which the serum of human beings injected with alcoholic extract of cultural *S. pallida* develops complement-fixing properties with *pallida*, but not with Wassermann, antigen) by a number of experiments on rabbits, in which, as they said, a Wassermann reaction can be provoked by injection of a variety of substances. Rabbits injected intravenously with alcoholic extract of washed cultural *S. pallida* gave a

reaction most strongly with *S. pallida* antigen, but some also with Wassermann antigen. Saturation of the sera with a suspension of *S. pallida* removed or weakened the reaction with *pallida* antigen but not with the Wassermann. Heating to 63° C. removed the reaction with Wassermann, but not with *pallida* antigen. Six general paretics with strongly positive Wassermann reactions, but none with *pallida* antigen were injected with *pallida* extract and then began to react with this antigen, but the Wassermann reaction did not increase in strength. Heating the sera to 63° C. removed the Wassermann, but, with some exceptions, not the reaction with *pallida* antigen. Like other workers, they observed the great variability of rabbit's serum, which changes from time to time without apparent cause.

In a later paper, Kroó and Schulze (1929) reported that the serum of human beings injected respectively with *pallida* vaccine of two strains developed complement-fixing properties which were strongly strain-specific, as has been mentioned in connection with spirochætolysins. They ended this paper with the argument that, if the Wassermann reaction depended on antibody to *S. pallida*, one would expect it to become stronger in secondary syphilis under treatment, owing to auto-inoculation with enormous numbers of *S. pallida*.

As regards strain-specificity, Georgi, Prausnitz and Fischer (1929) found that after accustoming a Noguchi strain of *S. pallida* cultivated on rabbit-kidney ascitic agar to grow in rabbit-brain ascitic agar, it provoked antibodies which were specific for brain-grown Noguchi *S. pallida* as distinct from the original kidney-grown strain, and from the Kroó strain of *S. pallida*. The authors suggested that a similar mutation of biological properties probably occurs in the case of *S. pallida* which become established in the parenchyma of the central nervous system.

Torii (1928) also carried out a number of experiments in an attempt to determine whether the Wassermann reaction depends on antibody to organ lipid or to spirochætal lipid, using injections of egg-yolk, various organ lipoids, and spirochætal lipoids. He found that the sera of animals treated with organ lipid gave the strongest Wassermann reactions, probably because of the variety of antigens in an alcoholic extract of organ such as is used in the Wassermann test. Using spirochætal and testicular lipoids as antigen in complement-fixation tests with syphilitic rabbits' sera, he obtained positive reactions practically only with the testicular lipoids. There was no striking difference in these tests between the reactions with syphilitic testicle lipoids and those with normal testicle lipoids. Torii believed, therefore, that the Wassermann reaction depends on antibody to the tissue lipoids, the role of *S. pallida* being that of an activator like the pig's serum in Sachs, Klopstock and Weil's experiments.

If the possibility of the auto-generation of antitissue-lipoids in the body can be accepted, it is natural to enquire if any specificity of reaction with lipoids of various tissues can be demonstrated. Georgi and Fischer

(1927) tested the sera of 102 human syphilitics with a special brain-extract as well as with the ordinary extract used in the Wassermann test. In general they found close parallelism between the reaction with brain-extract and changes in the spinal fluid. In nine sera and one fluid the reaction with brain-extract was positive while that with heart-extract was negative. On the other hand, in treated cases the brain-extract reaction was often negative while the fluid was still pathological. The authors remarked on the diagnostic possibilities suggested by the use of brain-extract for complement-fixation tests, but their results do not appear to have been confirmed.

Plaut and Kassowitz (1929) refer to the work of Brandt, Guth, R. Müller and Weil, and also of Heimann and Steinfeld, who found that the serum of rabbits inoculated with brain-lipoids plus pig-serum gave definite complement-fixation reactions in high dilution with brain-lipoid antigen, but only feeble or negative reactions with alcoholic extract of other organs. The brain-lipoid reaction, though specific for organ, was not specific for animal, as it occurred with brain-lipoids from various animals. Witebsky and Steinfeld had shown that injections of aqueous heterologous brain-suspension created in rabbits complement-fixing antisera which were specific for brain-lipoids and sometimes also for the type of animal when fresh, unboiled brain was used in the test; the type-specificity in these cases was attributed to the albumins in the antigen. Plaut and Kassowitz confirmed Witebsky and Steinfeld's results, except that they obtained no antiserum which was specific for the animal. Both complement fixation and organ-specificity were not so strong with watery as with alcoholic extracts. They inoculated some rabbits with rat-brain, and others with ox-brain and tested their sera for complement fixation with alcoholic extracts of a variety of organs, with and without cholesterol. In a few cases the antisera acted slightly with lipoids of other organs, but the specificity for brain preponderated strongly. Seven of the eighteen rabbits' sera did not react with alcoholic extract, but gave strong reactions with aqueous brain suspensions. These sera tended to fix complement with aqueous suspensions of other organs, especially kidney. Plaut and Kassowitz repeated the experiments on syphilitic rabbits which had become Wassermann-negative and obtained substantially the same results without the Wassermann reaction becoming again positive. On the other hand, inoculation of guinea-pigs and rats failed to produce similar antisera, results which are in accord with those of other workers, who have also failed in the case of man. Plaut and Kassowitz concluded that, until extensive experiments with organ-lipoid immunization have been made with animals other than rabbits, the behaviour in the rabbit must be regarded as exceptional and cannot be applied to man.

SUMMARY.

The work on serum-antibodies which has been reviewed above seems to reveal a striking difference between the antigenic properties of cultural

and infecting *S. pallida*. While it seems to be easy to provoke in rabbit or man antibody for cultural spirochaetes, this antibody has no effect on *S. pallida* infecting men and animals, and antibody to cultural *S. pallida* is not found in the serum of syphilitic rabbits or human beings. So far as it goes, also, the evidence is that neither experimental nor natural infection provokes the formation of antibody, apart from agglutinins, for any *S. pallida* whether cultural or otherwise. The difference in response seems to lend colour to the scepticism of Kast and Kolmer as to the identity of cultural *S. pallida* with that concerned in syphilis. The evidence as to the nature and origin of the properties or bodies in the serum which result in complement fixation and flocculation reactions is clearly insufficient for a decision.

REFERENCES.

- AUDRY, C., 1926, *Bull. Soc. franç. Derm. Syph.*, **33**, 26.
 BERGEL, S., 1928, *Klin. Wschr.*, **7**, 1681.
 BERNARD, R., 1926, *Brux. méd.*, **7**, 72, 105.
 BLUMENTHAL, G., 1929, *Z. Hyg. InfektKr.*, **110**, 93.
 BRANDT, R., 1922, *Wien. klin. Wschr.*, **35**, 223.
 BREINL, F. & WAGNER, R., 1929, *Z. ImmunForsch.*, **60**, 23.
 BROWN, W. H. & PEARCE, L., 1920¹, *Arch. Derm. Syph.*, N.Y., **2**, 675; 1920², *Amer. J. Syph.*, **4**, 593; 1920³, *Arch. Derm. Syph.*, N.Y., **2**, 470; 1920⁴, *Trans. Ass. Amer. Phys.*, **35**, 291; 1921¹, *Amer. J. Syph.*, **5**, 1; 1921², *Arch. Derm. Syph.*, N.Y., **3**, 254; 1921³, *Proc. Soc. Exp. Biol.*, N.Y., **18**, 255; 1921⁴, *J. Exp. Med.*, **34**, 185; 1922, *J. Exp. Med.*, **35**, 39; 1923, *Proc. Soc. Exp. Biol.*, N.Y., **20**, 476; 1924, *J. Exp. Med.*, **39**, 645; 1927, *J. Exp. Med.*, **45**, 497.
 BRUCK, C., 1928, *Klin. Wschr.*, **7**, 71.
 CHESNEY, A. M., 1927, *Medicine Monographs, Immunity in Syphilis*. Baltimore. London, Baillière, Tindall & Cox. pp. 12 and 20.
 CHESNEY, A. M. & KEMP, J. E., 1924, *J. Exp. Med.*, **39**, 553; 1925¹, *ibid.*, **41**, 479; 1925², *ibid.*, **42**, 17; 1926, *ibid.*, **44**, 589.
 CHESNEY, A. M., TURNER, T. B. & HALLEY, C. R. L., 1928, *Johns Hopk. Hosp. Bull.*, **42**, 319.
 COHN, A., 1929¹, *Klin. Wschr.*, **8**, 886; 1929², *ibid.*, 1310.
 CRAIG, C. F. & NICHOLS, H. J., 1912, *J. Exp. Med.*, **16**, 336.
 DOLD, H. & WORMS, W., 1928, *Klin. Wschr.*, **7**, 2140.
 EAGLE, H., 1930, *J. Exp. Med.*, **52**, 717, 739, 747.
 EASTWOOD, A., 1920, *Rep. Publ. Hlth. Med. Subj. Lond.*, No. 1, p. 78.
 EBERSON, F., 1921, *Arch. Derm. Syph.*, N.Y., **4**, 490.
 EBERSON, F. & ENGMAN, M. F., 1921, *J. Amer. Med. Ass.*, **76**, 160.
 FINGER, E. & LANDSTEINER, K., 1906, *SitzBer. Akad. Wiss. Wien*, **115**, 179.
 FORTIG, H., 1927, *Z. ImmunForsch.*, **52**, 328, cited by BLUMENTHAL.
 FREI, W., 1923, *Arch. Derm. Syph.*, Wien, **144**, 365.
 FREI, W. & GRÜNMANDEL, S., 1927, *Z. ImmunForsch.*, **51**, 517, cited by BLUMENTHAL.
 GAETGENS, W., 1929, *Med. Klinik.*, **25**, 390.
 GEORGI, F. & FISCHER, O., 1927, *Klin. Wschr.*, **6**, 2328 and 2423.
 GEORGI, F., FRAUSNITZ, C. & FISCHER, O., 1929, *Klin. Wschr.*, **8**, 2007.
 HALLEY, C. R. L. & WASSERMANN, H., 1928, *Arch. Intern. Med.*, **41**, 843.
 HASHIMOTO, T., 1926, *Brux. méd.*, **6**, 1362.
 HENNING, L., 1928, *Z. ImmunForsch.*, **55**, 19, cited by BLUMENTHAL.
 HERONIMUS, E. & AWRECH, W., 1927, *Z. ImmunForsch.*, **53**, 541, cited by BLUMENTHAL.
 HOELTZER, R. R. & POPOFF, W. J., 1928, *Z. ImmunForsch.*, **59**, 501.
 HOFFMANN, E., 1906, *Derm. Z.*, **13**, 565.
 HUNTER, J., 1810, *A Treatise on the Venereal Disease*, 3rd Edn., London, p. 312.
 JAHNEL, F. & LANGE, J., 1925, *Munch. med. Wschr.*, **72**, 1452; 1927, *ibid.*, **74**, 1487.
 JAUREGUY & LANCELOTTI, 1924, *Bull. Acad. Méd.*, Paris, **92**, 1295.

- KAST, C. C. & KOLMER, J. A., 1929, *Amer. J. Syph.*, **13**, 419.
- KISSMEYER, A., 1915, *Deuts. med. Wschr.*, **41**, 306; 1917, *ibid.*, **43**, 1447.
- KLOPSTOCK, F., 1926, *Deuts. med. Wschr.*, **52**, 226; 1928, *Klin. Wschr.*, **7**, 600, 1896.
- KOLLE, W., 1922, *Deuts. med. Wschr.*, **48**, 1301; 1924¹, *ibid.*, **50**, 1074; 1924², *ibid.*, 1235.
- KOLLE, W. & EVERS, E., 1926¹, *Deuts. med. Wschr.*, **52**, 557; 1926², *ibid.*, 1075.
- KOLLE, W. & PRIGGE, R., 1929, *Deuts. med. Wschr.*, **55**, 985.
- KOLLE, W. & SCHLOSSBERGER, H., 1928, *Deuts. med. Wschr.*, **54**, 129.
- KOLMER, J. A., 1913, *J. Exp. Med.*, **18**, 18.
- KROÓ, H. & SCHULZE, F. O., 1928, *Klin. Wschr.*, **7**, 246; 1929, *ibid.*, **8**, 1203.
- KROÓ, H., SCHULZE, F. O. & ZANDER, L., 1929, *Klin. Wschr.*, **8**, 783.
- LEVADITI, C., 1905, *C.R. Soc. Biol.*, Paris, **58**, 845.
- LEVADITI, C., LAROCHE, G. & YAMANOUCHI, T., 1908, *C.R. Soc. Biol.*, Paris, **64**, 720.
- MANO, Y., 1928, *Deuts. med. Wschr.*, **54**, 993.
- MANTEUFEL, P. & RICHTER, A., 1926, *Deuts. med. Wschr.*, **52**, 2113.
- MARIE, A. & LEVADITI, C., 1907, *Ann. Inst. Pasteur*, **21**, 138.
- MOORE, J. E., 1922, *Arch. Intern. Med.*, **30**, 548; 1923, *Johns Hopk. Hosp. Bull.*, **34**, 89.
- NEISSER, A., 1911, cited from CHESNEY & KEMP (1925²).
- NEUFELD, L. & SZYLE, D., 1928, *Z. Exp. Med.*, **60**, 355, cited by BLUMENTHAL.
- NICHOLS, H. J., 1911, *J. Amer. Med. Ass.*, **63**, 466.
- NICHOLS, H. J. & WALKER, J. E., 1923, *J. Exp. Med.*, **37**, 525.
- PEARCE, L., 1928, *J. Exp. Med.*, **47**, 611; **48**, 125 and 363.
- PLAUT, F. & KASSOWITZ, H., 1929, *Z. ImmunForsch.*, **63**, 428.
- PRIGGE, R., 1927, *Deuts. med. Wschr.*, **53**, 1340; 1928, *Arb. Inst. exp. Ther. Frankfurt*, No. **21**, 152; 1929, *Ther. Gegenw.*, **70**, 385, 447.
- PRIGGE, R. & ROTHERMUNDT, M., 1927, *Derm. Z.*, **50**, 169.
- PRIGGE, R. & RUTHOWSKI, E. v., 1929, *Deuts. med. Wschr.*, **55**, 1508.
- QUEYRAT, L., 1906, *Ann. Derm. Syph.*, Paris, **7**, 147.
- RACCHIUSA, S., 1921-2, *Ann. Clin. med.*, Palermo, **11**, 271, cited by BLUMENTHAL.
- REITER, H., 1928, *Klin. Wschr.*, **7**, 1539.
- SACHS, H. & GEORGI, W., 1918, *Med. Klinik.*, **14**, 805.
- SACHS, H. & KLOPSTOCK, A., 1926, *Deuts. med. Wschr.*, **52**, 650; 1927, *ibid.*, **53**, 394.
- SACHS, H., KLOPSTOCK, A. & WEIL, A. J., 1925, *Deuts. med. Wschr.*, **51**, 589.
- SCHARNKE & RUETE, 1921, *Z. ges. Neurol. Psychiat.*, Orig., **64**, 343.
- SCHLOSSBERGER, H., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 237.
- TOMASZCZEWSKI, E., 1910, *Berl. klin. Wschr.*, **47**, 1447.
- TORII, F., 1928, *Japan. J. Derm. and Urol.*, **28**, German Summary 43.
- UHLENHUTH, P. & GROSSMANN, H., 1928, *Z. ImmunForsch.*, **55**, 380.
- UHLENHUTH, P. & MULZER, P., 1913, *Arb. GesundhAmt.*, Berl., **44**, 307.
- UHLENHUTH, P. & WEIDANZ, O., 1908, *Deuts. med. Wschr.*, **34**, 862.
- VOEGLIN, C. & DYER, H. A., 1925, *Publ. Hlth. Rep.*, Wash., **40**, 2511; **42**, 176.
- WAKERLIN, G. E., 1926, *J. Infect. Dis.*, **38**, 323.
- WASSERMANN, A., NEISSER, A. & BRUCK, C., 1906, *Deuts. med. Wschr.*, **39**, 745.
- WILKES-WEISS, D., RICHTER, C. & KOLMER, J. A., 1926, *J. Infect. Dis.*, **38**, 378.
- WORMS, W., 1926, *Deuts. med. Wschr.*, **52**, 785.
- ZINSSER, H. & HOPKINS, J. G., 1915, *J. Exp. Med.*, **21**, 576.
- ZINSSER, H., HOPKINS, J. G. & MCBURNEY, M., 1916, *J. Exp. Med.*, **23**, 341.

Practical Diagnosis.

MICROSCOPICAL EXAMINATION.

In the case of chancres and secondary lesions of the skin or mucous membranes of the early papular type a diagnosis can readily be made by scraping the lesion and examining the exudate microscopically under dark-ground illumination. The surface of the lesion should previously be cleaned as well as possible in order to avoid confusion with surface

spirochætes, some of which may be very fine and *pallida*-like. If the chancre has almost healed or has been treated with antiseptics, its margin should be punctured rather deeply with a spud, or some juice be aspirated from a regional lymphatic gland. In the case of mouth lesions contamination with saliva should be avoided so far as possible, and a small ring curette, which both scrapes the lesion and picks up the specimen like a platinum loop, is useful because it lessens the chances of saliva flowing over the lesion after it has been mopped dry.

Under dark-ground illumination *S. pallida* is easily distinguished from other spirochætes found in genital lesions by the following characteristics : (1) it is a regularly coiled, silvery-white spiral with about seven coils to the width of a red blood cell ; (2) its coils are clear-cut ; (3) though it can be fairly active in its own ground, bending into loops and angles, it does not move rapidly from point to point. Other spirochætes likely to be found in secretions from this region are of three main varieties : (a) a short thick spiral of 2 or 3 turns, which races quickly across the field ; (b) a long thick spiral, which moves with moderate speed ; and (c) the only one likely to cause any difficulty in specimens from the genitals, a regularly coiled spiral about twice the thickness of *S. pallida*. It is not so flexible as *S. pallida*, and, like the other two spirochætes just mentioned, when the focus is shifted slightly upwards, it has a slightly rusty tinge, due doubtless to rays which have passed through its body and been refracted.

In specimens from the mouth and throat two spirochætes which are rather finer than *S. pallida* may be seen. One has more angular turns and the other has shallower spirals, rather like those of a piece of twisted silk.

The dark-ground method of microscopical examination is useful also for the detection of *S. pallida* in the organs of a macerated fœtus and in the brains of general paralytics.

In specimens stained by the Giemsa and Leishman methods, besides the regularity and fineness of the coils, the fact of *S. pallida* staining rose-pink instead of blue has some diagnostic value.

In experimental work with rabbits it is necessary to avoid the error of attributing to syphilitic inoculation the lesions resulting from infection with *S. cuniculi*. For example, Levaditi and Marie (1919) described anomalous lesions in rabbits following inoculation with the blood of a general parietic and inferred that they were dealing with a neurotropic strain of *S. pallida*. Their description is, however, so closely similar to those by Levaditi, Marie and Nicolau (1921), Noguchi (1922) and Nichols and Walker (1923) of infection with *S. cuniculi* as to make it extremely probable that they were dealing with this organism. The chief points by which infection with *S. cuniculi* can be distinguished from that with *S. pallida* are its tendency in the secretion of lesions to become agglutinated into tangled masses, its inability to develop in the testis, the non-infectivity of the glands, and the negative Wassermann reaction.

SERUM TESTS.

General Principles of Complement-Fixation Tests.

The origin of the complement-fixation test known as the Wassermann, or the Bordet-Wassermann, test for syphilis has been described (p. 231). The test depends on the fact that the sera of most persons in whom there is an active syphilitic focus, when mixed with alcoholic extract of a solid organ, fix more complement than do the sera of non-syphilitic persons, with the exception of those suffering from certain diseases which will be mentioned below. Some complement is fixed by extract alone, some by serum alone, and some by non-syphilitic serum plus extract, so that the test must be quantitative. The amount of complement fixed by an extract depends on the source and method of preparation of both. The amount fixed by a serum plus an extract depends on the extract, whether the serum has been heated or not, the amount of each and the conditions under which fixation proceeds. The aim is to devise a combination of reagents with which, in these proportions and conditions, the result will never be positive with a non-syphilitic or non-frambœsial serum, but be positive with the highest possible proportion of syphilitic or frambœsial sera. In the effort to achieve this end, a large variety of combinations has been devised by different pathologists.

The methods employed at present may be divided first into those in which the reagents are obtained from different sources and those in which the complement and anti-sheep-cell amboceptor (Hecht), or only the complement (Stern) present in the tested serum is employed.

Complement-Fixation Tests on the Principles of Hecht and of Stern.

Tests on the Hecht and the Stern principles can be disposed of quickly. Both afford higher percentages of positive reactions in syphilis than do those on the first-mentioned principle, but neither can be recommended except as a supplementary test, and in this role they have now been supplanted by the flocculation tests. On scientific grounds they are open to the objections that the complement and anti-sheep cell amboceptor in a human serum are variable in amount, that complement in different human sera varies in fixability and that unheated serum often contains thermo-labile substances which fix complement in the absence of syphilis. In so important a test the whole batch of sera tested on a given occasion should be exposed to conditions which are as nearly as possible identical. This is impossible with the methods under consideration, and such 'short-cut' methods should be used only by way of supplement to methods based on the principle that the reagents are derived from different sources and their properties can be tested independently.

Tests on the Principle of the Original Wassermann Reaction.

It would be impossible here to discuss in detail the different methods which have been evolved on the basis of the original Wassermann test, and only some of the outstanding principles underlying the better-known

ones can be mentioned. The discussion must also be limited to methods in which the hæmolytic system employed in the test for free complement is an emulsion of sheep's cells with anti-sheep's cell hæmolysin. Some prefer ox-cells and some human, but the majority employ sheep's cells.

The antigen.

The antigen is the constituent of the Wassermann test over the preparation of which there has been the greatest controversy. An extract of an organ is a highly complex product with three properties of importance in the Wassermann test, viz. antigenic, anticomplementary and hæmolytic. The first is desirable, the other two are undesirable. Unfortunately, by most methods of preparation an increase of the desirable is accompanied by some increase of the undesirable properties. The problem is to obtain the best compromise, and upon this there is much diversity of opinion.

The antigens most commonly used now are extracts of syphilitic liver and of healthy heart. Some workers test sera with both of these, but most of those who employ only one extract obtain it from heart. This practice, which is based on trial and error, has been substantiated by Kolmer (1928¹), who has shown that heart-extract contains more of the desirable and less of the undesirable than does liver.

The source of the heart-muscle does not seem to be of any great importance so long as the species is not heterogenetic (Forsmann) as it would then interact with the anti-sheep's cell hæmolysin present in most human sera and bind complement in the absence of antibody. Thus, human- or ox-heart is serviceable, if fresh and healthy, but not guinea-pig or horse since either of these tends, like sheep and dog's heart-extract, to afford non-specific reactions with sera containing anti-sheep-cell hæmolysin. Ruediger (1919) tested 50 sera from clinically non-syphilitic persons with antigens derived from heart respectively of man, ox, rabbit, sheep, dog and guinea-pig and obtained false positives with dog and guinea-pig extracts. Taniguchi (1920) found that a human serum which was rich in anti-sheep hæmolysin fixed five times as much complement with guinea-pig heart-extract as with human- or ox-heart, while another containing no anti-sheep hæmolysin fixed the same amount of complement with each of these three extracts.

Fortification of extract with cholesterol. Following on observations by Browning, Cruickshank and Mackenzie (1910) that the addition of cholesterol to a solution of lecithin improved its complement-fixing powers with syphilitic sera, Sachs (1911) recommended the addition of cholesterol to organ extracts. Fildes and McIntosh (1913), after an extensive series of tests of extracts of various organs with and without cholesterol, arrived at an optimum of two parts of 1 per cent. cholesterol and three parts of alcoholic extract of human heart. This combination, which is used in the McIntosh and Fildes method and in the modification elaborated by myself (Nos. 3 and 1 methods respectively in Medical Research Council's Special Report Series, No. 14), has proved satisfactory.

The mode of action of cholesterol in the test is uncertain, as it is, itself, not antigenic. Griffith and Scott (1920¹) likened the action of cholesterol to that of an intensifier in photography, while Browning and Mackenzie (1924¹), inclined to the view that the action is not due merely to its influence on the physical state of the emulsion, but that a chemical factor is also involved. The views of Eagle (1930), which support those of Griffith and Scott, were discussed on p. 232. Whatever the explanation, within limits, the greater the concentration of cholesterol in an extract-cholesterol emulsion the stronger the complement-fixing power.

The conditions under which fixation proceeds affect the proportion of cholesterol which it is safe to use. As pointed out by Jacobsthal (1910), when the serum-extract-complement mixture is kept in the ice-chest for a number of hours before the test for free complement is applied, there is eventually in most cases a greater fixation of complement than when the preliminary fixation occurs at 37° C. for 30 to 60 minutes. One factor contributing to this result is the deterioration of complement with age, another is the greater anticomplementary effect of all extracts (without serum) under the conditions of contact for several hours in the ice-chest. A concentration of cholesterol which does not afford non-specific results under fixation at 37° C. for one hour may do so under low temperature for 18 hours, so that in the cold fixation method less cholesterol must be used.

The variability of the cholesterol content of crude alcoholic extracts raises the suggestion that it would be better to remove it, so that different brews of extract of a given organ when fortified with a given proportion of cholesterol would be uniform in respect of this important constituent. A suggestion based on this principle was made by Griffith and Scott, who proposed to use an extract made according to the directions of Noguchi and Bronfenbrenner (1911) and then to add the optimum amount of cholesterol, as determined by experiment. Noguchi and Bronfenbrenner's extract is the ether-soluble, acetone-insoluble fraction of heart-muscle. The rejected acetone-soluble fraction contains the cholesterol and hæmolytic bodies which are undesirable. It is less sensitive than a crude heart-extract, but is practically free from anticomplementary properties. With preliminary fixation in the ice-chest over-night, Griffith and Scott found equal parts of Noguchi extract and of a 1 per cent. alcoholic solution of cholesterol yielded the most uniform results. They also found that the proportion of antigen to serum is important; a low dilution of antigen may give a negative result with syphilitic serum, while a high dilution may give almost complete fixation.

Kolmer (1928²), after considerable research, concluded that an ether extract contains less lipid and more undesirable hæmolytic constituents than does an alcoholic and is, therefore, inferior to this, and that there is little to choose between a cholesterolized acetone-insoluble (lecithin), fraction and a cholesterolized crude extract, but consideration of the

content of the respective antigens in undesirables—auto-anticomplementary and hæmolytic substances—showed an advantage in favour of the acetone-insoluble lipoids. With a 1 in 10 dilution of each extract acting alone, Kolmer (1928¹⁰) found that the same amount of complement was inactivated at 37° C. in one hour by much the same amount of the different extracts (extremes 0.95 and 0.80 c.cm.), but at 8° C. in 18 hours there was more difference, viz. from 0.80 c.cm. with the acetone-insoluble lipoids, to 0.50 c.cm. with the alcoholic extract of heart plus 0.4 per cent. cholesterol. He concluded that the safest antigen was one from which as much as possible of the anticomplementary and hæmolytic properties had been removed, and prepared one on the following lines: Dried, perfectly fresh, human- or ox-heart is extracted with ether, which removes some lecithin and most of the hæmolytic substances. The ethereal extract is kept for further treatment. The solid residue is dried and extracted with alcohol, which removes some antigenic and hæmolytic substances and much anticomplementary. This alcoholic extract is also reserved for further treatment. The solid is now left with a negligible content of anticomplementary and hæmolytic substances and some antigenic, all of which are extracted with absolute ethyl alcohol (apparently for 7 to 8 days). The ethereal and the first alcoholic extract are precipitated with acetone, the precipitates are dissolved in ether and are then added, with 0.2 per cent. cholesterol, to the second alcoholic extract. According to Kolmer, the dose of this extract employed in the test can be at least ten times the minimum required to fix the standard amount of complement with a pooled serum from six or more syphilitic individuals. This dose is no more than one-thirtieth of that which would fix the complement when acting alone. Table I, constructed from one by Kolmer, compares the three successive extracts and the finished product under the same conditions.

TABLE I.

Showing the hæmolytic, anticomplementary and antigenic properties of different fractions of Kolmer extract.

<i>Dilution in which 0.5 c.cm. was—</i>			
	<i>Hæmolytic.</i>	<i>Anti-complementary.</i>	<i>Antigenic.</i>
Primary ether extract ..	1 to 8	1 to 4	1 to 50
Primary alcoholic extract ..	1 to 4	1 to 16	1 to 20
Second alcoholic extract ..	1 to 2	1 to 10	1 to 125
Finished product	1 to 2	1 to 8	1 to 2,400

Extracts containing no cholesterol. Like Noguchi and Bronfenbrenner, some workers remove the cholesterol with acetone but add no cholesterol. A well-known example of this practice is the Bordet-Ruelens extract, which, according to Renaux's description, is now made by washing calf's heart with acetone until this is free from colour, drying the residue and

extracting with 95 per cent. alcohol for 10 to 12 days. It is made up for use by evaporating 1 c.cm. to dryness, adding 3 c.cm. distilled water and then 27 c.cm. of 0.9 per cent. NaCl.

According to Griffith and Scott, this method of suspension enhances the antigenic power of an extract in much the same way as does cholesterol. They found that when a suspension of Noguchi extract was prepared by the Bordet method its antigenic potency equalled that of a cholesterolized Noguchi extract diluted in the ordinary way. On the other hand, the Bordet method applied to a cholesterolized extract resulted in a suspension of diminished efficiency.

The tested serum.

Inactivation. In the original directions for the Wassermann test it was recommended that the serum should be heated to inactivate the natural complement. Later, it was found that unheated serum gave stronger reactions, and various methods employing unheated serum were devised. It was shown, however, by Sachs and Altmann and many others that unheated serum may give non-specific reactions. Browning, Dunlop and Kennaway (1922) found that one factor in this effect is the concentration of alcohol in the extract suspension. Thus, an unheated non-syphilitic serum with a 1 in 30 dilution of cholesterolized antigen allowed complete lysis with 2 m.h.d. complement, but when enough alcohol was added to raise its concentration to 1 in 12, the mixture fixed 6 m.h.d. complement. With some unheated normal sera alcohol alone in a concentration of 1 in 8 brought about a positive reaction.

Gilmour (1924) found that treating a negative serum for five minutes at 55° C. removed its non-specific reacting power. Heating a positive serum reduced the fixing property, and the loss was practically as great after five minutes as after an hour. Kolmer, Rule and Trist (1920) also found that heating at 55° C. for five minutes is sufficient to remove these non-specific substances, but, in disagreement with Gilmour's results, that heating syphilitic sera at 55° C. caused a progressive loss of reacting power up to one hour. This accords with my own observations (1910) which showed, as did Kolmer, Rule and Trist's, that the effect might not be very obvious with strongly positive sera, but was very marked with those from treated cases. Kolmer, Rule and Trist recommended heating the sera at 55° C. for only 15 minutes, as this was sufficient to dispose of the auto-anticomplementary property mentioned below.

Auto-anticomplementary properties of sera. Apart from non-specific reaction with extract, the serum may be unduly anticomplementary, or become so on keeping. In most cases this property is removed by heating the serum at 55° C. before the test. In those cases in which the property persists the cause may lie in (a) heavy contamination of the serum with bacterial growth; (b) the use of anti-sheep's-cell lysin derived from horse, this being heterogenous (Forsmann) for the anti-sheep-cell lysin in human serum. For a period, in the laboratory of my Department at

St. Thomas's Hospital, the proportion of sera found to be anticomplementary was a serious nuisance. After changing from horse *v.* sheep's-cell lysin to rabbit *v.* sheep's-cell the occasions on which the serum-control failed to show complete hæmolysis became very rare.

Leprosy sera have been found by Thomsen and Bjarnhjedinson (1910) and others to be naturally anticomplementary. Others believe that syphilitic sera have this property, but it has certainly not been very apparent in the thousands of tests which have passed under my review. Kolmer (1928³) says that placenta and cord-blood should be carefully watched from this point of view. The possibility of a serum being anticomplementary naturally demands the inclusion of a serum control in the test.

For sera from which the anticomplementary substances cannot be removed by heat, Sachs (1921) recommended addition of 8.2 c.cm. N/300 HCl to each c.cm. serum, allowing to stand for 30 minutes, centrifuging and removing the supernatant fluid, to which is added 0.8 c.cm. of 10 per cent. NaCl solution.

Hæmoglobin in the serum may interfere with the test by making its reading more difficult. Apart from this, it may be an indication that the specimen has been contaminated with traces of spirit used for sterilization of needles and syringes. Wyler (1927) showed that, if 5 c.cm. blood is added to 0.3 c.cm. spirit (which produces very little hæmolysis), the effect on the reaction is a quite definite weakening, which might convert a just-positive serum into a negative.

Anti-sheep's-cell lysin in human sera. The presence in most human sera of a variable amount of anti-sheep's-cell lysin is a factor of some importance, apart from its bearing on the choice of animal from which the organ extract is obtained.

In the test as usually practised the amount of complement-serum employed is that containing two to three times the minimum amount of complement which would hæmolyse the dose of cells plus hæmolysin added at the end ; it varies with the hæmolytic activity of the complement-serum employed on the day of the test and must be determined by titration. Strictly speaking, the procedure is not scientifically sound because the hæmolytic activity of a complement-serum does not run parallel with the fixability, but it works reasonably well. The hæmolytic activity (m.h.d.) of a complement-serum varies, within limits, with the amount of hæmolysin with which the sheep's cells are sensitized, so that, although one may add to the dose of sheep's cells in each tube exactly the same quantity of rabbit *v.* sheep's-cell hæmolysin, the sensitization of the cells in the tubes becomes different when they are brought into contact with the different human sera in a batch of tests, by reason of the variable amount of man-*v.*-sheep's-cell hæmolysin in these sera. The effect may be that with a given amount of complement hæmolysis might be complete in some tubes and incomplete in others, and the results would therefore be uneven.

Two remedies suggest themselves: (a) to remove beforehand the anti-sheep's-cell lysin from the sera under test, or (b) to sensitize the sheep's cells so fully that the additional lysin in the human sera becomes of no moment.

The first method is practised by Kolmer, who adds to the human sera whilst still on the clot one drop of a suspension of washed sheep's red cells for each c.cm. of the serum; places the mixture in the cold for 15 minutes, centrifuges, and finally inactivates the separated serum at 55° C. for 15 minutes. Previous removal of the anti-sheep's-cell lysin from the serum is necessary in Kolmer's method of the Wassermann test because he uses only 2 m.h.d. of added hæmolysin and 2 m.h.d. complement. Kolmer and Rule (1920) found that, in the Kolmer method, removal of the natural lysin resulted in 3 to 5 per cent. more positive reactions and in 6 per cent. giving stronger reactions.

The method of sensitizing the cells fully so that the natural hæmolysin becomes of no moment has always appealed to me as simpler and perhaps more likely to result in uniformity of the hæmolytic system throughout a batch of tests. It is effected by adding not less than 5 m.h.d. anti-sheep's-cell lysin to the cells. Griffith and Scott (1920²) found the m.h.d. of complement very slightly lower with cells sensitized with 10 m.h.d. hæmolysin, but for practical purposes it can be taken that, if the cells are sensitized with 5 m.h.d., any additional hæmolysin they may meet in the serum under test will not affect the activity of the complement. Maximum sensitization has the further advantage of reducing to a minimum the amount of guinea-pig serum to be placed in each tube. Thus, Kolmer (1928⁴) found that while 0.15 c.cm. of a dilution of guinea-pig serum may be required to hæmolyse a given dose of cells sensitized with 2 m.h.d., only 0.09 c.cm. may suffice to hæmolyse the same quantity sensitized with 5 m.h.d., equivalent in an actual test in which 3 m.h.d. complement are used to 0.45 c.cm. of 1 in 30 guinea-pig serum if the cells are sensitized with 2 m.h.d. and to 0.27 c.cm. if they are sensitized with 5 m.h.d. In most cases the greater the amount of guinea-pig serum required to provide the number of m.h.d. used in the test the greater the likelihood of hæmolysis in the final result, so that, other things being equal, a worker who uses 2 m.h.d. complement with 2 m.h.d. hæmolysin may easily show more negative results than one who uses 3 m.h.d. complement with 5 m.h.d. hæmolysin. The importance of the quantity of guinea-pig serum used is generally well recognized by workers, who discard complement which is comparatively inactive because it affords too many negatives. Equally they discard complement which is unduly active because it is apt to afford non-specific reactions, but the occasions on which this is necessary are infrequent. A good example of admission that it can be dangerous to be tied too closely to the complement titre in determining the amount of guinea-pig serum to be added is found in Kolmer's advice (1928⁵) that, in his test, if the complement titration shows 0.1 c.cm. of 1 in 30 guinea-pig serum to be the m.h.d., the

amount to be used in the test should be, not 0.2 c.cm. (containing the 2 m.h.d. commonly used in Kolmer's method), but double this amount.

The complement-serum.

The complement has already been discussed in its relation to other reagents. It has been shown by Browning and Mackenzie (1924²) that the fixability of complement from different guinea-pigs varies. They found that a serum removed from the clot very quickly after taking the blood was more fixable than after it had stood in the ice-chest for 24 hours, though the m.h.d. had not changed. Griffith and Scott (1920³) found that, amongst 60 guinea-pigs, four yielded complements which were too sensitive for use in the Wassermann test, and this characteristic persisted for a full year; autopsy in one case revealed no disease.

Pregnancy, even in its earliest stage, lowered the hæmolytic power and raised the sensitiveness. For this and other reasons they recommended the use of only healthy, well-grown male animals.

As illustrating the variations in fixability of different complements, they occasionally found that a complement which was sensitive to antigen alone was unduly insensitive when in the presence of antigen plus serum, possibly owing to the serum protecting it from the antigen. In other cases a complement which was weak in lytic power and insensitive to antigen alone was hypersensitive to antigen plus serum. Again, of two complement-sera differing in hæmolytic power and used in equal amounts with the same syphilitic serum and antigen, the more lytic might be more readily fixed. In such a case an attempt to compensate for the greater hæmolytic power by using less complement-serum would only increase the divergence of the results.

Variability of complements was better demonstrated when the strength of reaction of a positive serum was tested by grading the complement. Thus, of two complements equal in lytic power and in sensitiveness to antigen alone that were tested with the same serum and antigen, twice as much of the one was fixed as of the other. On the other hand, when a series of dilutions of human serum were tested, the same amount of the two complement-sera was fixed by a dilution of 1 in 40. The inference appears to be that, for a fine gradation of the strength of the Wassermann reaction, it is better to employ different dilutions of serum with constant complement than vice versa. I adopted the complement gradation method for routine work because it seemed easier, but in special cases in which I have wished to find the end-point of the reaction have preferred the method of serum gradations.

Griffith and Scott found that grading of serum even did not always compensate for irregularity of complements, and that pooling of complement, which is practised by many workers, did not overcome the difficulty. A positive serum diluted 1 in 20 gave with six different complements reactions ranging from completely positive to almost

completely negative. The same serum gave two-fifths lysis with a mixture of three of the complements, one-fifth lysis with a mixture of four of them and practically no lysis with a mixture of all six.

The use of a constant amount of complement-serum. Griffith and Scott concluded from their experiments that, since the determination of the hæmolytic titre of a complement-serum, even in the presence of antigen, cannot be relied upon to produce uniformity of results, it would be better to use a fixed amount of guinea-pig serum (one volume of a 1 in 25 dilution), requiring only that its hæmolytic activity (complete lysis of one volume of sensitized cells by one volume of a 1 in 31 dilution) and sensitiveness to antigen alone should lie within certain limits. They proposed to use with this complement an antigen containing a fixed amount of cholesterol and highly diluted (1 in 640) so that it shows anticomplementary power with only exceptional specimens of complement. There is much to be said for the proposal, and it seems surprising that it has not attracted more attention considering the illogicality of determining the dose of complement-serum by its hæmolytic titre.

Preservation of complement. The variability of complement and the desirability of obtaining results which are comparable from one week to another suggests that a reliable method of preserving it would be a boon. In 1911 I adopted the plan of keeping it frozen hard, and this method has proved satisfactory for keeping complement for a week or so. Salting has been satisfactory in many workers' hands. Friedberger (1907) recommended the addition of pure sodium chloride to a strength of 4 per cent. I found that this method worked well for at least five weeks. Tulloch (1928) recommends what is perhaps the best method, viz. addition of NaCl to a concentration of 9.9 per cent. and then drying by the Hartley, Eagleton and Okell (1923) method. The dried product is weighed and the weight related to the volume of the original. If 0.5 gm. = 3.3 c.cm. of the original, a 1 in 12 dilution is obtained by taking it up with 39.6 c.cm. distilled water. Addition of magnesium sulphate followed by drying, addition of glycerin, and of sodium acetate have also been found successful. Kolmer (1928^a) has found that addition of 0.25 gm. NaCl to each c.cm. serum and keeping it in the ice-chest at 0 to 4° C. is a satisfactory method of preserving complement for three to four weeks.

Order of addition of reagents.

The order in which the reagents are mixed may be of some importance in tests of weakly acting sera. Dean (1911), in bacterial complement-fixation tests, showed that, if serum and antigen are long in contact before the addition of complement, there is less fixation than when the three are added in rapid succession. Griffith and Scott (1920³), in Wassermann tests, found that only when the antigen was minimal and antigen and serum were in contact at 37° C. for an hour before addition of complement could any effect of this kind be demonstrated. In spite of the slight influence suggested by Griffith and Scott's experiments that the order

of addition might exercise under ordinary working conditions, it would seem best, for the sake of weakly acting sera, to avoid bringing serum and antigen into contact in the absence of complement.

Preliminary fixation in the cold versus fixation at 37° C.

As already mentioned, fixation in the cold for several hours increases the total fixation, and various workers, including Kolmer, recommend it in preference to fixation at 37° C. A disadvantage is the practical one that it prolongs the time taken over the test ; another one is that, as pointed out to me by Dr. Osmond, some complements seem to deteriorate more rapidly than others whilst standing over-night in the ice-chest. Otherwise, it is probable that, by suitable adjustment of proportions of reagents, as safe and sensitive a test can be made under fixation in the ice-chest as at 37° C. Gilbert, Langworthy and Moore (1926) found that, with a cholesterolized antigen and all reagents in the same proportions, four hours' fixation at 3 to 6° C. gave more positive reactions than did fixation for 30 minutes at 37° C. As regards incubation at 37° C., this may be carried out in an incubator or in a water-bath at this temperature, remembering that the rise of temperature in the former is slower than in the latter, so that half an hour in the water-bath is probably equivalent to an hour in the incubator. As combining the advantages of cold and warm incubation, leaving the mixtures at room temperature for half an hour followed by half an hour in the water-bath may be mentioned, and has been found to work well in practice.

The hæmolytic system.

The hæmolytic serum and the cells have already been considered in relation to the treatment of the serum under test. It is agreed that a high-titre hæmolysin should be used. The cells should be well washed and the suspension of uniform concentration from week to week. Wyler (1929) has found standardization of the cell suspension by the Bigger method (1921) of CO-hæmoglobin estimation practical and satisfactory. Naturally, concentration of the cells, like degree of sensitization, affects the amount of guinea-pig serum added. Since the cells serve only the purpose of an indicator of the presence of free complement, and the more guinea-pig serum added the less sensitive the test, I have always advocated the use of a 3 per cent. suspension. Also, for the above and other reasons already stated, I have sensitized them fully before commencing titrations, preparing enough for the complete day's work, so that, so far as the cells are concerned, the conditions are equal for titrations and the actual tests. Kolmer adds cells and lysin separately and sensitizes with only two doses as he finds that fully sensitized cells give in his hands more negative results. Wyler (unpublished) has compared the Kolmer method with his modification of the Medical Research Council No. 1 method in which the principle of full sensitization is employed, and has found that, when the complement titre in the Kolmer method is read rather on the low side, as Kolmer recommends, the No. 1 method is more delicate ; when it is

read on the high side (i.e. using rather less complement in the test) it is approximately equal in sensitiveness to the No. 1 method, with a slight advantage in sensitivity in favour of the latter.

Questions of this kind can be settled only by technicians meeting and testing in one laboratory a large number of the same sera (as adopted in the two laboratory conferences at Copenhagen in 1923 and 1928), working in close collaboration with sound clinicians in order to ascertain that the results agree with the clinical data.

Tests Depending on Directly Visible Effects of Interaction between Serum and Extract.

Precipitation reactions of various kinds began to be published shortly after the appearance of the Wassermann test, but did not become established as a practical method until the publication of the Sachs-Georgi test in 1918. Meinicke (1918, 1919, 1920) also devised a flocculation method based upon an increased turbidity produced by addition of unheated syphilitic serum to an alcoholic extract of horse-heart greatly diluted with alcohol and containing balsam of tolu and benzoic acid. This was succeeded in 1929 by the Meinicke Klärungs-Reaktion (M.K.R.).

The Sachs-Georgi test. Lentochol and citochol methods.

In 1918 Sachs and Georgi published a flocculation test which has attracted wide attention and has formed the basis of large numbers of modifications and refinements. It depends on the formation of flocculi in a mixture of inactivated syphilitic serum and a heart-extract diluted with alcohol suitably cholesterolized and suspended in saline. The tubes are incubated for 18 to 24 hours at 37° C. and the results read through an agglutinoscope. More recently, Sachs and Witebsky (1928¹ & ²), stimulated by the success of Kahn's test (reviewed below), have devised a test which can be carried out very quickly. The alcoholic extract of ox-heart used in their original (now called 'Lentochol') test is evaporated to dryness, and the residue taken up with three parts of alcohol. To this concentrated extract is added 0.3 to 0.6 per cent. cholesterol (the optimum for the particular extract having been determined by experiment). The cholesterolized extract is diluted with two parts of 0.85 per cent. NaCl, and to 0.1 c.cm. of the emulsion is added 0.2 c.cm. inactivated serum. The mixture is stirred for 5 to 10 seconds and then left at room temperature for half an hour, after which 1.0 c.cm. saline is added, and the results are read either with the naked eye or through an agglutinoscope (fractions of the quantities mentioned can be used). The new ('Citochol') test in Sachs and Witebsky's hands proved more delicate than the Lentochol at the Copenhagen Laboratory Conference in 1928, but not so delicate as the Kahn or Müller.

The Sigma test.

Dreyer and Ward (1921) elaborated a test, the Sigma, on the principle of the Sachs-Georgi Lentochol test, but differing from it in the greater precision of its technique and in being much more finely quantitative.

In some thousands of tests of the same sera by Wyler it has been found to be more delicate than No. 1 Method (M.R.C.) of the Wassermann test, especially in treated cases, and the same was found in a comparison with the McIntosh and Fildes method of the Wassermann [No. 3 Method (M.R.C.)] which was carried out on 894 sera by Dreyer and Ward on the one hand and McIntosh and Fildes on the other. My observation of this test as carried out by Wyler and other workers for the Health Organization of the League of Nations, both in their own laboratories and in the Laboratory Conferences held in Copenhagen, have impressed me with the vital importance of adhering strictly to the authors' technique. Such modifications as I have witnessed have yielded results far behind those mentioned above.

The Kahn test.

Kahn (1922) has devised a test which has been adopted exclusively by the U.S. Naval Department and has attracted widespread interest. It is based on principles detailed in *The Kahn Test—A Practical Guide* (p. 26).

The antigen is an alcoholic extract of beef-heart from which the ether-soluble fractions have mostly been removed by previous treatment of dried heart-muscle with ether. Cholesterol is added to make a concentration of 0.6 per cent. The antigen is standardized to discover the smallest amount of salt solution which when added to 1 c.cm. of antigen produces aggregates capable of complete dispersion on addition of further salt solution; usually the amount ranges from 1.0 to 1.5 c.cm. per c.cm. of antigen. The antigen is tested for sensitiveness against syphilitic sera, and if different from the standard is adjusted with the help of sensitizing reagent (cholesterolized alcoholic solution of the dried residue resulting from evaporation of an ethereal extract of heart) and of cholesterolized alcohol, or by evaporation to increase its lipid concentration. Since publication of Kahn's guide, the method of standardization has undergone some changes as a result of further work by Kahn, Lubin and McDermott (1929). In the test proper, three different amounts (0.05, 0.025 and 0.0125 c.cm.) of an optimum antigen suspension (say, antigen 1, saline 1.1) which have stood for 10 minutes are put into test-tubes, and to each is quickly added 0.15 c.cm. inactivated serum. Each tube is at once well shaken for ten seconds, and later, when all the tests have been put up, all are shaken in a machine for three minutes. Then 1 c.cm. saline is added to each tube containing 0.05 c.cm. antigen and 0.5 c.cm. to each of the others, after which the results are read. Kahn distinguishes six types of reaction: (a) Four positive, ranging from four-plus to one-plus for various degrees of flocculation, from large particles easily seen in a clear medium down to very fine particles in a somewhat turbid medium; (b) doubtful, with extremely fine particles only just visible; and (c) negative, with opalescent medium which is free from particles. The author has devised a presumptive test of syphilis with an antigen which is more sensitive than the standard.

Its negative might be regarded as a strong evidence against, but its positive only as presumptive, and not decisive, evidence of syphilis. The routine test in the hands of its author proved more delicate than any of the fourteen methods compared at the Copenhagen Conference in 1928 with the exception of Müller's, and it gave no positive reaction with non-syphilitic sera. In other hands it may not have proved so delicate or reliable. Kolmer (1928⁷) shows the results afforded by the same sera (issued by the New York Public Health Laboratory) in his laboratory with the Kolmer Wassermann and in Kahn's laboratory with the Kahn test. They are reproduced in Table II. Thus, as found with the Sigma and other precipitation tests, the Kahn proved more sensitive than the

TABLE II.

<i>Stage.</i>		<i>Sera.</i>	<i>Per cent. positive.</i>	
			<i>Kahn.</i>	<i>Kolmer.</i>
Primary syphilis ..	..	24	58	67
Secondary syphilis ..	..	6	100	100
Tertiary syphilis ..	..	24	54	71
Neurosyphilis ..	..	20	80	70
Congenital syphilis ..	..	30	37	40
Treated syphilis ..	..	105	47	44

Wassermann with sera from treated cases. Table III, reproduced from Kolmer's work, shows the results obtained by other workers with the same sera. They support the impression derived from a study of the voluminous literature on the subject that in less experienced hands the test is neither so delicate nor so reliable as it appeared in Kahn's hands at the Copenhagen Conference in 1928.

TABLE III.

Results of tests by the Kahn method obtained by different workers with the same sera.

<i>Laboratory.</i>	<i>Syphilitic sera.</i>		<i>Controls.</i>	
	<i>Per cent.</i>		<i>Per cent.</i>	
	<i>Positive.</i>	<i>Unsatisfactory.</i>	<i>Positive.</i>	<i>Unsatisfactory.</i>
Illinois State Department of Health	31.7	0	0	0
Western Reserve University	51.2	13.9	6.9	23.4
Michigan State Department of Health (Kahn's) ..	43.6	0.4	0	0

Slide precipitation tests.

The Kahn test has occasioned the birth of a number of rapid tests, one of which, the Citochol of Sachs and Witebsky, has been mentioned.

The M.K.R. can be carried out on a microscope slide either when the amount of serum is too small for a full test in tubes, or when a result is required quickly (the tube test requires 18 to 20 hours' incubation). In

this case a drop of the mixture from one of the tubes is placed on a microscope slide and this in a damp atmosphere for an hour, when the result is read under the microscope.

Kline and Young (1927) devised a modification of the Kahn test in which the reaction is observed under the microscope. Since the first publication of this test, Kline (1928) has made considerable changes in the preparation of the antigen (originally as used in the Kahn test), from which he claims to have removed much that reacts with non-syphilitic sera. His method of preparing the extract emulsion differs radically from that usually practised. The cholesterol is first precipitated with distilled water, and the precipitate is then dispersed by addition of extract followed by energetic shaking for thirty seconds and then by saline. Two emulsions are prepared, standard and very sensitive, the latter containing more cholesterol, and the emulsions remain stable for three days. In the test of an inactivated serum the mixtures, two for each specimen, are made in a pair of thin wax rings formed on a microscope slide, 3 in. by 2 in., each slide being prepared for six tests. When a dozen tests have been put up the pair of slides, in a simple wooden frame, are rotated on a flat surface for four minutes and the mixtures are then examined under a magnification of about a hundred. A positive reaction has much the same appearance as in a bacterial agglutination, so that reading is very easy. Kline (1929) has also adapted the test for whole blood obtained by a finger prick. In this case the blood is first defibrinated with the help of a spicule of wood and a measured drop of it placed in the centre of a thin wax ring on a slide as above. A drop of 7 per cent. NaCl is added and then a drop of a very sensitive emulsion (prepared rather differently from that for the serum test) after which the slide is rotated for four minutes. The blood is then laked by addition of distilled water, the mixture overflowing the inner ring into a moat, the outer wall of which is a thick wax ring previously formed round the thin one. In this test, as in that for unheated serum (Kline, 1930), the pH of the distilled water used in making the salt solution should be 5·4 to 6·0.

The whole blood test is used in Kline's laboratory for transfusion donors, its speed of application making it particularly suitable for this purpose. In several thousands of tests the Kline method has proved equal in simplicity, sensitivity and specificity to the best of the others.

Ring test.

The Murata modification of the Sachs-Georgi test depends on the formation of a ring at the junction of inactivated serum with a 1 in 10 dilution of an ox-heart extract to which has been added an optimum amount of 1 per cent. cholesterol solution (1 per cent. cholesterol to 17·5, 18, 18·5 or 19 extract) as discovered by preliminary trial with normal and syphilitic sera. The diluted extract is floated on the top of the serum, and the tubes stand for 45 minutes at room temperature. The test is thus a very simple one and quickly performed. At the Copenhagen

Conference in 1928, it afforded 251 positives with 491 syphilitic sera (Kahn, 299; Müller, 310; M.T.R. 241), and two positives with 426 non-syphilitic (Kahn, 0; Müller, 1; M.T.R. 9).

Müller's 'Ballungs Reaktion' (M.B.R.) or conglobation test.

Müller (1925) depends on the formation of a single globular, white or yellowish white, gelatinous mass in a mixture of inactivated syphilitic serum and an emulsion of a specially prepared, cholesterolized ox-heart extract. The preparation of the extract and of its emulsion demands special care, and the author's directions must be followed very precisely. More recently, Müller (1929) has published a modification (M.B.R. II) which is simpler and has the advantage that the result is ready in 3 or 4 hours, as compared with 18 or 20 hours required by M.B.R. By a further modification (1930) in which the mixtures are centrifugalized (after being heated at 56° C. for 15 minutes) the time period has been shortened still further, and strongly positive reactions may be apparent in 30 minutes. Both M.B.R. and M.B.R. II are as easily read as a Wassermann, and M.B.R. proved the most sensitive of all the methods compared at the Copenhagen Conference in 1928.

Stern and Frank (1930), in a recent comparison of the Wassermann, M.T.R., the Citrochol and Lentochol S-G.R. and the M.B.R. methods, found the order of specificity to be W.R.—M.K.R.—M.B.R.—Lentochol—M.T.R.—Citrochol; and the order of delicacy, M.K.R.—Citrochol—M.B.R.—M.T.R.—W.R.—Lentochol. They concluded that the M.K.R. is the best supplementary test to the W.R.

The Hinton glycerol cholesterol test.

Hinton (1927, 1929) has devised a test which depends on agglutination of cholesterol. He uses as extract the ether-insoluble, alcohol-soluble fraction of beef-steak muscle diluted with 9 parts of 0.7 per cent. solution of cholesterol in alcohol. One part of this extract is diluted with 2 parts of 5 per cent. NaCl solution and shaken, after which 12 parts of 5 per cent. NaCl solution are added, and, after further shaking, 15 parts of 50 per cent. solution of glycerin. The inactivated serum is put up in amounts of 0.1, 0.3 and 0.5 c.cm., and to each is added 0.5 c.cm. of the antigen which has been prepared as above. In the case of a syphilitic serum, after incubation at 37° C. for 16 to 18 hours the cholesterol particles are clumped. For spinal fluids the procedure is similar but the amounts of fluid are 2.0, 1.5, 1.0, 0.5 and 0.1 c.cm. The test as shown to me by Dr. Hinton seems easy to read. Cheever and Splaine (1928) tested 2,036 specimens in parallel by the Wassermann, Kahn, Kline and Young's slide precipitation and Hinton methods, and found the Hinton the most sensitive, though it gave no more non-specific reactions than any of the others. Splaine and Cheever (1928) found the Hinton method only slightly better than the Wassermann in primary cases, but 50 per cent. more sensitive in latent ones.

The Vernes test and syphilimetry.

Vernes (1920) measures the turbidity in a mixture of serum and his extract, called 'péréthynol', by means of a photometer and gauges the state of the patient by fluctuations of the degree of reaction. Péréthynol is made from horse's heart by treatment of the powdered muscle with perchloride of ethylene and with absolute alcohol. The important operation of preparing the colloidal suspension is carried out with an electrically driven machine and a special technique. In the test the reagents are distributed with a special syringe (Vernes's 'rhéomètre') which automatically measures the required amount. Corresponding to each mixture of inactivated serum and extract suspension is a control of the serum mixed with a solution of alcohol in water, the dilution (1 to 6·5) equalling that of the extract. The mixtures are incubated in a water-bath at 25° C. for 4 hours, when the degree of turbidity of each pair is read with the photometer. The difference between the turbidity of the serum-antigen mixture and that of the control is the degree of reaction, which may range from 0 to 150. Most normal sera are said to give a reaction varying between 0 and 3 on the scale; a few give a higher reading up to 10. In the absence of undoubted evidence from other sources, a diagnosis of syphilis is founded on changes in the degree of reaction afforded by specimens of serum taken at intervals of some days. A non-syphilitic serum is said to remain at approximately the same degree as that shown in the first test; a syphilitic serum fluctuates widely. The behaviour of the curve representing degrees of reaction at different times is believed to be an index of the activity of the infection, and Vernes has coined the term '*Syphilimetry*' for the measurements of this activity by means of his test. As a guide to treatment, for example, if the degree of reaction remains steady, this may be regarded as an indication to change the remedy. Used as a test of cure, when the degree of reaction has been reduced to the base line, treatment is suspended and the serum is tested eight times at monthly intervals. If the reaction remains steadily on the base line and the cerebrospinal fluid is normal, the disease is believed to have been eradicated. With a patient whose spinal fluid is normal, but whose syphilis has not been eradicated an upward curve invariably occurs within eight months of the last injection of an arsenobenzene preparation.

A legitimate criticism is that the Vernes test is not so delicate as others. At the Copenhagen Conference in 1928 in the hands of its author and his colleague, M. Bricq, with 448 sera from syphilitic cases only 171 results were recorded as positive against 277 by Kahn, and 190 by No. 1 method (M.R.C.) of the Wassermann test. These results accorded generally with those obtained by Wyler (unpublished) before the Conference. It was not possible to repeat the tests on specimens of the same cases so as to demonstrate the oscillations in the degree of reaction on which Vernes insists as the criterion of a syphilitic infection, but the observations serve to show that the Vernes test is not so ready to respond to syphilitic changes

in the serum as are some other methods. Commenting on the results obtained at the Conference, Vernes complained that many of the sera tested there were not in a suitable state for his test ; in fact, he regarded 187 of the 448 sera tested as unsuitable because they were either chylous or granular. He requires that sera should be not more than 3 or 4 days old. A practical disadvantage of the method appears to be the fatigue of reading results by the photometer ; it also requires somewhat expensive apparatus.

Diagnostic Value of the Wassermann and Flocculation Tests of the Blood-Serum.

These should be considered in three cases—the person over the age of three months, the new-born infant and the rabbit.

Serum reactions after the age of three months.

Significance of negative reactions. As a general rule, the serum of a syphilitic person gives a negative reaction to any test until about ten days after appearance of the primary lesion. After this the percentage of positive reactions rises rapidly until at the time when the generalized symptoms are due the reactions are positive in practically every case. At this time such a test as the Sigma, which measures the strength of the reaction very closely, usually shows a higher number of units, sometimes several hundreds, than at any other period during the course of the infection. In later stages with external manifestations a good test, either Wassermann or flocculation, usually gives a positive reaction. In the presence, therefore, of manifestations of disease outside the central nervous system, negative Wassermann and flocculation reactions can justifiably be regarded as strong evidence that those manifestations are non-syphilitic. At the same time, and this is, after all, the important point in considering the individual case, negative reactions are not absolute evidence against a diagnosis of syphilis. The clinician who refuses to consider a diagnosis of syphilis because the serum reactions are negative will sooner or later find that he has committed a grave error for which his patient may suffer. Negative serum reactions are fair evidence that manifestations of disease in the central nervous system are non-syphilitic, but in a small proportion of cases the fluid is positive though the serum is negative, and, in practice, an examination of the cerebrospinal fluid should not be omitted.

The value of negative serum reactions in the absence of manifestations of disease depends naturally on the percentage of latent cases which give a positive reaction, and this is unknown. Judging by tests of untreated mothers of congenital syphilitics, persons, that is, in whom the existence of a syphilitic infection is reasonably certain, the reactions tend naturally to weaken with age of the infection, since the proportion of positive reactions in untreated latent syphilitic women is in the region of 75 per cent. It may be expected to be higher in men because serum reactions

depend on tissue reaction, and this, as Warthin (1928) has shown in autopsy material, and clinical experience agrees, is stronger in men than in women.

A negative reaction obtained during or shortly after a course of treatment is useless for determining the question of eradication of the disease. In my experience, however, when the reaction returns to positive, this occurs in the majority of cases within the first year following suspension of treatment and is rare after two years. Whether or not this means that most persons whose reactions (including those of the cerebrospinal fluid) following treatment have remained negative for two years are rid of the disease is by no means certain. Warthin (1929¹) has never seen at autopsy a healed case of syphilis and, if this applies to all cases, the value of negative reactions, no matter how long after suspension of treatment, is nil so far as concerns the affirmation of eradication of the disease. Short of this, however, assuming, as seems justifiable, that the serum reaction reflects the reaction of the tissues to the virus and therefore in most cases the activity of the latter, negative reactions obtained after administration of treatment should be some index of its effect, and this seems to be borne out by clinical experience. For example, patients whose serum reactions become negative quickly under treatment usually, though not invariably, do well, and in our experience it is mainly the positive reactors who suffer from later manifestations of syphilis.

Since the better flocculation tests afford a higher percentage of positive reactions in treated cases than do complement-fixation ones, a negative flocculation test is naturally more significant than a negative Wassermann.

It should be remembered that, as noted by various workers, the reactions in latent syphilis may fluctuate between positive and negative independently of treatment. In one case observed at the Rochester Row Hospital, they were alternately negative and positive to tests carried out weekly for a number of weeks.

Significance of positive reactions. The value of definitely positive reactions in affirming the presence of syphilis may conveniently be discussed under the headings of the Wassermann and the flocculation tests respectively since by no means all workers place so much reliance on a positive flocculation reaction as on a positive Wassermann. The significance attached to a positive Wassermann reaction raises the question of the non-syphilitic diseases in which the serum has afforded such reactions. Besides yaws, which may be accepted at once, and relapsing fever, which should in any case cause no trouble in diagnosis, a large variety have been reported at one time and another. They include many febrile conditions such as scarlet, typhoid, typhus and especially malarial fevers and pneumonia, also tuberculosis, malignant disease, leprosy, pregnancy and a number of dermatoses, such as urticaria pigmentosa, scleroderma, psoriasis and pityriasis rosea, as well as such conditions as diabetes mellitus, uræmia and jaundice.

My own experience applies to comparatively few of these. At the Laboratory Conferences at Copenhagen in 1923 and 1928 over 150 sera from cases of tuberculosis, over 180 from pregnant women and over 40 from cases of tumour were tested by most of the workers, and only those from syphilitic cases gave positive reactions to good methods.

As regards *malaria*, reports are discordant and confusing. My own views rest firmly on the results of an investigation carried out at my instigation by Thomson and Mills (1919) on 130 cases of malaria infected in all parts of the world and in all stages. The sera were tested by No. 1 method (M.R.C.), and eight positives were recorded. Each of these cases examined by Mills was found to be syphilitic. These results agree with those of numerous workers and lead to the conclusion that, provided the method is sound, a careful investigation of any case giving a positive reaction will usually reveal evidence of a syphilitic infection. It seems probable that a febrile attack in a latent syphilitic with negative serum reactions may temporarily stir up the syphilitic element. Certainly in paretics and tabetics the symptoms are often temporarily aggravated by malarial fever.

Leprosy sera, according to Schüffner (1912) and others, react with watery extracts but not with alcoholic. The results obtained by various workers leave one with much the same impression as that on positive reactions in malaria, namely that, in those cases where the reaction has been found to be positive, syphilis has not been excluded. Pineda and Roxas-Pineda (1926), after testing 650 lepers, concluded that, when the Wassermann reaction in such cases is clearly positive, it has the same significance as in non-lepers. Partial positives were obtained in a certain number of cases. In this connection the observation of Thomsen and Bjarnhjedinson (1910) that leper serum is very anticomplementary is significant.

In certain *dermatoses*, such as sclerodermia, psoriasis, pityriasis rosea and urticaria pigmentosa the sera may, in my experience, give partial reactions, and for this reason when using the No. 1 method (M.R.C.) I have never counted as positive a reaction in which there was not practically complete fixation. I have seen a number of cases of these kinds which have been diagnosed as syphilitic on the strength of reactions termed 'weakly positive', but proved on enquiry to be what I would term 'doubtful'. For this reason the term 'weakly positive' seems objectionable when applied to a reaction which the pathologist does not regard, for practical purposes, as diagnostic.

Altogether, so far as serum from a person over the age of three months is concerned, provided that the test is by a reliable method of the Wassermann and has been repeated on two specimens, my own views on the significance of a positive reaction coincide with those of Browning and Mackenzie (1924³), as follows: 'With reservations which have little importance in temperate climates, a positive reaction is a specific sign of syphilis and it indicates that the infection is, or has been till very lately, alive in the patient's tissues'. Similar views are expressed by

Kolmer (1928⁸), who accepts yaws and possibly relapsing fever but not trypanosomiasis, as liable to give positive reactions to a good method of the Wassermann test.

On the specificity of the various flocculation tests opinions differ more widely. The Sachs-Georgi, Sigma, Kahn, Kline, M.B.R. and M.K.R. tests have shown in good hands a degree of specificity which entitles them to rank with the Wassermann test for reliability on their positive reactions in the diagnosis of unknown cases. At the same time, I would personally not place quite as much reliance for diagnosis on a just-positive flocculation test as on a just-positive Wassermann, because I have seen more divergent results with the same flocculation tests in different hands than with the same methods of the Wassermann test. In a flocculation test a small departure from the proper procedure may make a very great difference in the result, as may also an error in a difficult reading. In a properly conducted Wassermann test on the other hand, there are more opportunities for checking the behaviour of the reagents step by step, and the test generally does not seem so sensitive to small divergencies from the right path.

The difficulty is, of course, solved by the generally recommended practice of testing each specimen by both a complement-fixation and a flocculation method. This has the further advantage that a higher percentage of positive reactions is obtained than by employing only one method, since a small percentage of sera react with the Wassermann but not with a good flocculation test, while the latter gives a higher percentage of positives with the sera of treated cases.

Reactions of sera from new-born infants.

Fildes (1915) showed in an examination of the cord-blood of 1,015 infants of viable age that no reliance can be placed on the serum reactions of the new-born infant for a decision on the question of its infection. The reactions of 7 which had been positive at birth were negative at 2½ to 4 months, while of 660 found to be negative at birth, 3 were positive on re-examination a few months later. In this view Whitridge Williams (1920) agreed. Out of 29 infants who were positive at birth, 7 became negative and showed no sign of syphilis, but of those who were negative at birth and were examined later, 10 became positive. Cruickshank (1922) came to the same conclusion. In 95 per cent. of 400 cases the reactions of mother and new-born infant were the same. In a series of 181 infants examined from 10 to 20 months later all who had reacted negatively continued to do so, but most of those who had given a positive reaction became negative. It seems clear that in most cases the reaction of the new-born infant only reflects the state of the mother, and a positive mother does not necessarily transmit syphilis to her offspring. On the other hand, a mother who is negative may bear a syphilitic infant, and if the infection of this is only recent at the time of birth, the reaction may not have had time to develop.

Serum reactions of rabbits.

As already mentioned, rabbit's serum is liable to give variable Wassermann reactions, a negative sometimes becoming positive after injection of lipoids and foreign proteins, and non-specific reactions to both complement-fixation and flocculation tests are not infrequent. Kolmer (1928⁹), in a review of the subject, mentions large variations depending upon the technique employed, so that one technique may give a negative and another a positive with the same serum, and heating the serum to 55° C. for half an hour increased this tendency. Kolmer found that by increasing the amounts of complement and hæmolysin used in the test more constant results are obtained. He also found that the negative or positive reaction obtained with a given technique remains constant during the life of the animal. Kemp, Chesney and Poole (1926), employing a special technique, obtained only two positives among 600 rabbits tested. In these the cause seemed to depend upon an anticomplementary action of the serum, and two weeks later the reactions became negative. Contrary to other workers, they did not obtain positive reactions in certain infections such as snuffles, coccidiosis, and subcutaneous abscess, from which some of their animals suffered. Infection with *S. cuniculi* gave negative reactions, as had been found by Noguchi (1922), Levaditi, Marie and Nicolau (1921), and Nichols and Walker (1923¹).

Saito (1929) tested 59 non-syphilitic rabbits by the Wassermann, using extracts of syphilitic liver, ox-heart, and cultural *S. pallida* respectively, and by the Kahn flocculation method, the serum being diluted 1 in 5. All the extracts gave some positives—38, 26 and 27 respectively—but the Kahn method gave none (with undiluted serum the Kahn gave 25 positives). In a repetition of the tests, the complement-fixation reactions varied greatly, but the Kahn reactions remained constant with one exception. Saito observed that a considerable proportion of the rabbits obtained from one source gave positive reactions, but most of those supplied by another dealer were negative. In rabbits inoculated with syphilis, the Kahn test seemed to be a more accurate guide to the course of the infection than any of the complement-fixation methods employed.

In rabbits inoculated with syphilis, Kemp, Chesney and Poole (1926) observed that the Wassermann reaction might be positive as early as two days after the appearance of the first clinical sign of infection. When the inoculum was small and the incubation period lengthened, the appearance of the reaction was delayed, depending apparently on the amount of tissue reaction. As a rule the serum reaction remained as long as the initial lesion, and it persisted longer in animals inoculated with the Nichols' strain, which produces more pronounced and persistent tissue reactions than other strains employed. In a number of infected animals in which the disease was allowed to run its course, the maximum strength of the reaction was attained in from two to six weeks after the appearance of the initial orchitis, persisted at this level for from two to three weeks

and then slowly declined to negative by about the hundredth day. The connection between reaction and tissue changes was demonstrated in rabbits castrated after the serum reaction had become positive. After castration the positive reaction promptly became negative, but on the appearance of further lesions the negative reaction reverted to positive. In animals treated with arsphenamine shortly after the appearance of a positive reaction (forty-first day of infection) this rapidly became negative, within three or four days after a single dose, but in those treated later (110 and 168 days after inoculation) the reversal to negative was tardy and often did not occur until a second dose was given three weeks after the first one. In two rabbits a negative reaction was provoked to positive by a dose of arsphenamine. In 8 animals treated early and cured the reaction became positive after reinoculation, though in 6 of them no primary lesion followed the reinoculation. In 7 of the reinoculated rabbits cross-inoculation proved them to have been reinfected. In rabbits treated 193 days after inoculation and proved to be non-infective, reinoculation was not followed by a positive reaction. In general, there was a close parallelism between a positive serum reaction and infectiousness of the lymph glands.

These experiments indicate that *with a suitable technique* the Wassermann test is a useful aid to the observation of results of experimental inoculation of rabbits, and also show the close dependence of the serum reactions on tissue changes.

TESTS OF THE CEREBROSPINAL FLUID.

Complement Fixation.

The Wassermann test of the cerebrospinal fluid is now performed with quantities of fluid five to ten times greater than those employed when testing serum. This practice, introduced by Hauptmann and Hössli in 1910, has resulted in the discovery of a considerably higher percentage of positive reactions than was reported at first. The fluid is not usually inactivated as it is anticomplementary only when contaminated with bacteria. Heating does not reduce the reacting power to nearly the same extent as does heating serum. Fildes and McIntosh (1913) found that it did not reduce the strength of the reaction in general paresis and tabes, and in cerebrospinal syphilis the reduction was only slight. White and Barber (1919) drew attention to the greater importance in Wassermann tests of the spinal fluid than in those of serum of titrating the complement in the presence of antigen in order to detect and to avoid the use of any complement so found to be abnormally sensitive to antigen alone. A non-syphilitic serum often protects such a complement, but spinal fluid does not seem to have this power.

General paresis, in the experience of most workers, has afforded a higher percentage of positive reactions and with smaller amounts of fluid than has any other form of neurosyphilis. The results collected by Browning

and Mackenzie (1924⁴) in 647 cases show 91 per cent. positive, and the same proportion with the serum. A more accurate idea is afforded by cases in which the diagnosis was verified by autopsy. Mott (1916) reported the percentage in over 300 such cases as 98·1. The percentage of positive reactions in the fluid of general paresis is thus so close to 100 that, for practical purposes, a negative reaction in any case presenting active symptoms and suspected of general paresis demands a careful revision of the diagnosis.

In *tabes* the percentage of positive reactions is much lower, and commonly larger amounts of fluid are required to elicit a reaction. Browning and Mackenzie's collection of results by various authors shows 54 per cent. positive in the fluid and 57 per cent. in the blood of 476 cases. This collection must include a number of stationary and treated cases, as in progressive cases it is uncommon to see a negative reaction. Fildes and McIntosh had 2 negatives in 24 cases, but in both the disease was quite stationary.

In *cerebrospinal syphilis* with active signs the reaction is usually positive. Fildes and McIntosh obtained only 4 negatives in 33 cases, but of these, 3 had been treated intensively. Browning and Mackenzie's collection showed 80 per cent. positive in 161 cases. Various workers have found that in cases with signs attributable only to endarteritis the reaction may be negative. This can be understood if it is agreed that the reaction depends on syphilitic changes in tissues, and while on the one hand a considerable degree of meningeal reaction may lead to comparatively slight symptoms, the blockage of a minute vessel may cause very pronounced ones.

Flocculation Reactions.

In general the flocculation methods have been believed to be less delicate tests of the spinal fluid than is the Wassermann, but this view requires revision. After the Copenhagen Conference in 1928 a comparison of the Kahn with the Wassermann method was organized in London, Kahn conducting his test and Wyler the Wassermann (No. 1 Method, M.R.C.). The specimens were divided into two series, cloudy and clear respectively, according to their appearance at the time of testing. In 238 specimens of cloudy fluids Kahn reported 48·1 per cent. positive and 5·5 per cent. doubtful to Wyler's 51·3 and 12·5 respectively, while in 317 clear specimens Kahn reported 46·9 per cent. positive and 5·1 per cent. doubtful to Wyler's 44·1 and 15·7 respectively. Generally, therefore, there seemed to be little difference between the two tests. In treated cases of syphilis (in which a doubtful reaction is regarded as significant) Kahn reported as positive or doubtful 53·2 per cent. in the cloudy series and 54·6 per cent. in the clear to Wyler's 60·6 and 64·5 respectively. In treated cases, therefore, the advantage seemed to be definitely with the Wassermann. When all factors are considered, there does not seem

to be much to choose between the two tests from the point of view of delicacy.

The Kahn test of spinal fluid is perhaps more troublesome to carry out than is the Wassermann, at any rate in the case of weakly acting specimens. After centrifugalization to remove the solids, the globulins in the supernatant fluid are precipitated with ammonium sulphate, separated as completely as possible, and are then redissolved in physiological saline, using an amount of this which is one-tenth the original volume of the fluid. Kline's test is applicable to the spinal fluid and easier to perform than the Kahn. Müller claimed verbally at the Second Copenhagen Conference that in the case of spinal fluid the M.B.R. is at least as delicate as the Wassermann. This is supported by the results of the 86 tests by both methods carried out at the Conference, as also by Engerth (1928) and others. M.B.R. II is equally applicable to the spinal fluid. Vernes prepares his antigen suspension differently for tests of fluid, the amount of fluid in proportion to suspension is greater than in the case of serum, and the degree of turbidity is read twice, viz. immediately and after four hours' incubation at 25° C. The difference between the two readings is the measure of the reaction. As judged by observations at the Copenhagen Conference in 1928, the test is more delicate than the Wassermann, but readings from 7 to 10 were recorded in 7 out of 22 fluids from non-syphilitic cases tested by this method. Such readings are said to be very exceptional (1 per 2,000) in the case of non-syphilitic sera.

The Sachs-Georgi and the Sigma are generally agreed to be rather less delicate tests of spinal fluid than is the Wassermann, and this is supported by results of tests at the Copenhagen Conference and by Wyler.

In the case of the M.T.R. reaction, Untersteiner (1925) and Schnitzer (1929) have introduced modifications for the examination of spinal fluid. The latter, by the modified method, obtained results which agreed fairly well with those of the Wassermann, but the positive reactions in syphilis were fewer and non-specific reactions occurred. Schnitzer was not able to make the reaction with M.T.R. extract more sensitive and after experimentation with various indicators evolved a new technique ('T.T.R.'), using colloidal turpentine C II of the Saxony Serum Works and alcoholic extract of syphilitic liver as prepared for the Wassermann test. Out of 100 fluids giving positive Wassermann reactions, 96 gave reactions to the T.T.R. Out of 244 which gave negative Wassermann reactions, 6 gave positive T.T.R.; 3 of these were syphilitic and 3 non-syphilitic.

Cytological Examination.

Most workers regard 0 to 5 cells per c.mm. as within normal limits 6 to 9 as doubtful, and 10 or more as pathological, though not necessarily syphilitic. Many think that the pathological limit should be lower than 10. Thus Kafka (1915) and others put it at 5, and Bloch and Vernes (1913)

consider more than one as pathological. Warwick and Nixon (1920), in a study of 805 spinal fluids from 408 patients, found no non-syphilitic or non-neurological case with a count of over five. Although the number of cells in neurosyphilis is usually above the normal limit, there does not seem to be any definite relation between the count and the nature of the syphilitic process. Further, as observed by numerous workers, the count may vary within wide limits from time to time. This might be due to settling or otherwise of the cells in the spinal canal, but Saunders and Spiegel (1928) did not find significant differences between specimens taken by lumbar puncture and those taken from the same subjects at the same time by cisternal puncture.

The findings in different stages of syphilis have already been discussed (p. 212).

Supplementary Reactions.

A large number of other phenomena associated with the cerebrospinal fluid have been utilized in the practical diagnosis of neurosyphilis, but since these are more in the nature of clinical aids than specific reactions they need not be referred to in detail in a work of this sort.

Protein content.

An increase of protein is usually found in the cerebrospinal fluid in neurosyphilis with or without symptoms. Many methods have been devised for demonstrating this, for instance Nonne and Apelt (1908) disclosed the globulins by half saturation with ammonium-sulphate and the albumins by boiling with acetic acid. A high globulin content usually coincides with a positive Wassermann reaction, but many exceptions are found to this rule.

Lange's colloidal gold test.

Lange (1912) introduced a test of the spinal fluid which is extensively used in routine diagnostic work. It depends on the work of Zsigmundy, who showed that colloidal solutions of gold are precipitated by electrolytes, but that some proteins protect them from this action. Lange adopted the reagent as a test for changes in the proteins of the spinal fluid in various pathological states and found that, in certain circumstances, the fluid from general paralytics precipitated the gold in the presence of 0.4 per cent. NaCl. A series of dilutions of the spinal fluid are mixed with the gold solution and the colour changes observed. According to the situation in the series of the chief colour changes, the reactions are described as Zone I (paretic), Zone 2 (luetic) and Zone 3 (meningitic). The paretic is so called because it is given by almost all cases of general paralysis and taboparesis, but it is not diagnostic of paresis, as it has been found in other forms of neurosyphilis and in some non-syphilitic conditions, particularly disseminated sclerosis. In tabes the reaction is usually of the luetic type.

In view of the difficulty formerly experienced in making the gold solutions other substances were introduced to serve a similar end, hence the colloidal-mastic test of Emanuel (1915) and the colloidal benzoïn test of Guillain, Laroche and Lechelle (1921).

Acetic anhydride test.

Boltz (1923) published a reaction in which the spinal fluid is mixed with acetic anhydride and emulsified. Sulphuric acid then produces a lilac colour in a positive case. Some authors have claimed a considerable degree of specificity for this test.

A consideration of the results of the supplementary reactions noticed above leads to the conclusion that only the Wassermann and flocculation tests are reliable in the diagnosis of neurosyphilis, since the others may give positive reactions in a variety of other affections of the central nervous system.

Effect of Treatment on the Different Pathological Reactions of the Cerebrospinal Fluid.

Provocative.

A temporary increase of the strength of pathological reactions of the fluid, or the appearance of such shortly after the institution of anti-syphilitic treatment, was found by Gennerich (1913) and has been reported by numerous workers, who regard it as similar in nature to the well-known Herxheimer reaction, which may occur in clinical symptoms and in serum reactions after institution of treatment. Fildes and McIntosh (1913) illustrated this effect in the case of the Wassermann reaction of the spinal fluid after injections of neosalvarsan, and believe that the provocative test is probably of greater value in neurology than in general syphilitic work, since in the former are more conditions resulting from very slight lesions which may fail to provoke a Wassermann reaction. Altmann and Dreyfus (1913) and Klauder (1919) demonstrated it in the fluid of primary and secondary cases. Solomon and Klauder (1920) published some cases in which it occurred after intravenous and intraspinal therapy. Warwick's (1921) series contained some cases in which the gold reaction and sometimes the globulin and the Wassermann were first evoked by treatment.

Eliminative.

The effect of treatment in reducing or abolishing pathological changes in the fluid naturally varies with the amount and nature of the changes. Fildes and McIntosh (1913) found as regards the Wassermann reaction that this is rapidly influenced in lymphovascular forms of syphilis and that resistance to treatment might be taken as some indication of the presence or absence of 'parasyphilis'. With (1917) found that generally in early meningovascular cases, and perhaps to a less extent in later ones, the reactions were often reduced or abolished, but in general paresis and tabes, although the cell count was reduced with fair ease, the Wassermann

reaction was most resistant. Kyrle (1920) agreed that in the secondary stage it is fairly easy to abolish pathological changes in the fluid ; indeed, if slight, they often disappear spontaneously. In later cases, however, he did not believe it possible with ordinary specific treatment to abolish them. He examined the fluid of 610 prostitutes, of whom 195 had never been treated, and of these, 94 showed changes in the fluid (slight in 35). Of the remaining 415, most of whom had been treated energetically and for a number of years, either with mercury or with arsenobenzene and mercury, 195 had pathological fluid (changes slight in 76). The close similarity between the proportions with pathological fluid in the treated and the untreated respectively seems to support Kyrle's view that, once pathological changes in the fluid are established, they are not abolished, though they may be reduced, by ordinary methods of treatment. The condition appears to be similar to that found in the blood-serum ; when the infection is of many months' standing the first course of treatment frequently reduces the strength of the reactions to a weaker, but still definitely positive, degree, around which they fluctuate with comparatively slight variations in spite of long continued further treatment.

The good clinical effects of malarial treatment on general paresis are generally admitted, but opinions are divided as to effects on the spinal fluid. Nicole and Steel (1925) re-examined the fluids of 25 general paretics after treatment by malaria and found improvement in gold and globulin reactions in most of them, more than half now giving a negative globulin reaction and a luetic gold reaction. These favourable results were obtained eleven or more months after the malaria, supporting the view of many workers that, after this form of treatment, improvements in the fluid changes are slow in making their appearance. Nicole and Steel show, like many others, that there is no reliable parallel between changes in the fluid and the clinical condition. O'Leary and Brunsting (1930) reported on the fluids of sixty patients with early general paresis inoculated with malaria between June, 1924, and June, 1926. In 52 per cent. of these the fluid had become negative, and in 30 per cent. it had improved to weakly positive Wassermann and positive Nonne, with cells within normal limits. In tabes and taboparesis the results had not been so good, but in 7 out of 10 cases of asymptomatic neurosyphilis observed for more than three years the fluid had become normal. O'Leary and Brunsting, however, also commented on the lack of parallelism between clinical and fluid changes.

THE RELATION BETWEEN SERUM AND SPINAL FLUID REACTIONS.

It is agreed that there is no true parallel between the reactions of the serum and those of the fluid. This applies especially to treated cases since treatment in early cases at any rate has a greater effect on the serum than on the spinal fluid. Persistently positive reactions of the serum after one or two courses of treatment are believed by Moore (1928) to suggest neurosyphilis in early cases, but in late ones they are more likely to be due to bone or visceral changes.

CUTANEOUS TESTS.

The introduction of cutaneous tests for tuberculosis stimulated Meirowski, Neisser and Bruck, Jadassohn and others to look for a similar test for syphilis. Noguchi (1911, 1914) found that when a suspension of killed cultural *S. pallida* was injected intradermally into syphilitics a cutaneous reaction ensued. The reaction was infrequent in the early stages, very constant in tertiary cases, and generally absent in non-syphilitics. Numerous other workers confirmed Noguchi's results, e.g. Robinson (1912), A. Brown (1913), Nanu-Muscel, Alexandrescu and Friedman (1914), De Buys and Lanford (1916). Several preparations for this cutaneous test have been placed on the market—luetin, pallidin (Klausner, 1914), syphilin, treponemina, Busson's organ luetin, Kroó's spiro-test (Joseph, 1929). These stock preparations are not, however, always reliable (see Alderson on luetin, 1922).

Various fallacies with this test have also been pointed out. Sherrick (1915) found that potassium iodide provoked a positive reaction which persisted for a considerable time (Kolmer and Broadwell, 1916), bromides and nitrates were found by Cole and Parýzek (1917) to have a similar, but less lasting, effect, and Strickler (1921) observed that the percentage of positives is increased if the subjects had been treated with arsphenamine. Stokes (1917) and Busacca (1922) also found that non-spirochætal or syphilitic substances, e.g. agar and gelatin, injected intradermally might excite local allergic reactions in certain syphilitic subjects.

Cutaneous tests have also been proposed as a means of *prognosis*. Horse-serum was introduced for this purpose by Dujardin and Decamps, and has been favourably reported on by Gougerot (1928) and Póvoa (1929). From what has been said it will be realized that the cutaneous test is hedged about with so many limitations and fallacies that it cannot occupy a strong position as a diagnostic or prognostic measure.

POST-MORTEM EXAMINATION.

The histological changes produced by syphilis in the tissues have been discussed (p. 204). Warthin has shown that very prolonged examination may be necessary before a subject can be pronounced to be non-syphilitic since all lesions may be microscopic. In males, Warthin (1929¹) has found the heart, aorta, central nervous system and the testicle to be by far the most frequent sites of lesions. In women, gross lesions are less likely to be found than in men, and then, according to Warthin (1928), more probably in liver, pancreas, adrenals and rectum. As regards the procedure, the examination of serial sections for characteristic lymphocytic infiltration and endarteritis, with resort to confirmation by Warthin's (1929) latest silver-starch-gelatin method for the demonstration of spirochætes in single tissue sections, appears the most likely to be successful.

The changes of value in the recognition of syphilis in the macerated foetus were discussed on p. 216.

REFERENCES.

- ALDERSON, H. E., 1922, *Arch. Derm. Syph.*, N.Y., **5**, 610.
 ALTMANN, K. & DREYFUS, G., 1913, *Münch. med. Wschr.*, **60**, 464.
 BIGGER, J. W., 1921, *Lancet*, Lond., ii, 1369.
 BISGAARD, A. H., 1910, *Mschr. Psychiat. Neurol.*, **28**, 124; 1913, *Z. ges. Neurol. Psychiat.*, **15**, 508.
 BLOCH, M. & VERNES, A., 1913, *C.R. Soc. Biol.*, Paris, **75**, 319.
 BOLTZ, O. H., 1923, *Amer. J. Psychiat.*, **3**, 111.
 BOYD, W., 1912, *J. Ment. Sci.*, **58**, 203.
 BROWN, A., 1913, *Amer. J. Dis. Child.*, **6**, 171.
 BROWNING, C., CRUICKSHANK, J. & MACKENZIE, I., 1910, *J. Path. Bact.*, **14**, 484.
 BROWNING, C., DUNLOP, E. M. & KENNAWAY, E. L., 1922, *J. Path. Bact.*, **25**, 36.
 BROWNING, C. H. & MACKENZIE, I., 1909, *Z. Immunforsch.*, Tl. I, Orig., **2**, 459; 1924¹, *Recent Methods in the Diagnosis and Treatment of Syphilis*, London, Constable, 2nd Edn., p. 341; 1924², *ibid.*, p. 140; 1924³, *ibid.*, p. 254; 1924⁴, *ibid.*, p. 317; 1924⁵, *ibid.*, p. 313.
 BUSACCA, A., 1922, *Wien. klin. Wschr.*, **35**, 523.
 CHEEVER, A. W. & SPLAINE, R. L., 1928, *New Engl. J. Med.*, **199**, 967.
 COLE, H. N. & PARYZEK, H. V., 1911, *J. Amer. Med. Ass.*, **68**, 1089.
 CRUICKSHANK, J., 1920, *Brit. J. Exp. Path.*, **1**, 71; 1922, *Brit. Med. J.*, ii, 593.
 DEAN, H. R., 1911, *Proc. R. Soc. Med.*, **5**, Path. Sect., 62.
 DE BUYS, L. R. & LANFORD, J. A., 1916, *Amer. J. Dis. Child.*, **12**, 387.
 DREYER, G. & WARD, H. K., 1921, *Lancet*, Lond., i, 956.
 DREYER, G., WARD, H. K., MCINTOSH, J. & FILDES P., 1923, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. **78**.
 EAGLE, H., 1930, *J. Exp. Med.*, **52**, 717, 739, 747.
 EMANUEL, G., 1915, *Berl. klin. Wschr.*, **52**, 792.
 ENGERTH, G., 1928, *Deuts. med. Wschr.*, **54**, 1374.
 FILDES, P., 1915, *Rep. Loc. Govt. Bd. Publ. Hlth.*, n.s., No. **105**.
 FILDES, P. & MCINTOSH, J., 1913, *Brain*, **36**, 193.
 FITZGERALD, E. J., 1930, *Brit. Med. J.*, i, 219.
 FORSSMAN, J., 1926, *Biochem. Z.*, **177**, 243.
 FRIEDBERGER, E., 1907, *Berl. klin. Wschr.*, **44**, 1299.
 GENNERICH, W., 1913, *Die Liquorveränderungen in den einzelnen Stadien der Syphilis* (Berlin).
 GILBERT, R., LANGWORTHY, M. S. & MOORE, A. C., 1926, *Amer. J. Syph.*, **10**, 162 and 506.
 GILMOUR, Ref. BROWNING & MACKENZIE, 1924, *Recent Methods in the Diagnosis and Treatment of Syphilis* (London), Constable, 2nd Edn., p. 157.
 GOUGEROT, H., 1928, *Amer. J. Syph.*, **12**, 487.
 GREENFIELD, J. G. & CARMICHAEL, E. A., 1927, *J. Neurol. Psychopath.*, **7**, 220.
 GRIFFITH, F. & SCOTT, W. M., 1920¹, *The Complement-Fixation Test in Syphilis*, *Rep. Publ. Hlth. Med. Subj.*, Lond., No. 1, p. 49; 1920², *ibid.*, p. 21; 1920³, *ibid.*, p. 68.
 GUILLAIN, G., LAROCHE, G. & LEHELLE, P., 1921, *Pr. méd.*, **29**, 773; 1922, *La réaction du Benzoin colloidal et les réactions colloïdales du liquide céphalorachidien*, Paris, Masson et Cie.
 HARRISON, L. W., 1910¹, *Power & Murphy's System of Syphilis* (London), Oxford Med. Pub., **6**, 185; 1910², *ibid.*, **6**, 252.
 HARTLEY, P., EAGLETON, A. J. & OKELL, C. C., 1923, *J. Path. Bact.*, **26**, 53.
 HAUPTMANN, A. & HÖSSL, H., 1910, *Münch. med. Wschr.*, **57**, 1581.
 HINTON, J., 1927, *Boston Med. Surg. J.*, **196**, 993; 1929, *J. Lab. Clin. Med.*, **14**, 621.
 JACOBSTHAL, E., 1910, *Münch. med. Wschr.*, **57**, 689.
 JOSEPH, A., 1929, *Klin. Wschr.*, **8**, 981.
 KAFKA, V., 1915, *Münch. med. Wschr.*, **62**, 105.
 KAHN, R. L., 1922, *Arch. Derm. Syph.*, N.Y., **5**, 570, 734, **6**, 332; 1925, *Serum Diagnosis of Syphilis by Precipitation*, Baltimore, Williams & Wilkins Co.; 1928, *The Kahn Test. A Practical Guide*, Baltimore, Williams & Wilkins Co.
 KAHN, R. L., LUBIN, G. & McDERMOTT, E., 1929, *J. Lab. Clin. Med.*, **14**, 876, 979.
 KEIDEL, A. & MOORE, J. E., 1921, *Arch. Neurol. Psychiat.*, Chicago, **6**, 286.
 KEMP, J. E., CHESNEY, A. M. & POOLE, A. K., 1926, *Johns Hopk. Hosp. Bull.*, **39**, 132.

- KLAUDER, J. V., 1919, *Amer. J. Syph.*, **3**, 559.
- KLAUSNER, E., 1914, *Münch. med. Wschr.*, **61**, 73.
- KLINE, B. S., 1928¹, *J. Lab. Clin. Med.*, **13**, 588; 1928², *Proc. Soc. Exp. Biol.*, N.Y., **25**, 689; 1929, *J. Lab. Clin. Med.*, **14**, 764; 1930, *ibid.*, **15**, 686.
- KLINE, B. S. & YOUNG, A. M., 1927, *J. Lab. Clin. Med.*, **12**, 477.
- KOLMER, J. A., 1928¹, *Serum Diagnosis by Complement Fixation* (London), Baillière, Tindall & Cox, p. 185; 1928², *ibid.*, p. 179; 1928³, *ibid.*, p. 98; 1928⁴, *ibid.*, p. 169; 1928⁵, *ibid.*, p. 393; 1928⁶, *ibid.*, p. 148; 1928⁷, *ibid.*, p. 479; 1928⁸, *ibid.*, p. 473; 1928⁹, *ibid.*, p. 344; 1928¹⁰, *ibid.*, p. 186.
- KOLMER, J. A. & BROADWELL, S., 1916, *J. Immunol.*, **1**, 429.
- KOLMER, J. A. & RULE, A., 1920, *Amer. J. Syph.*, **4**, 135.
- KOLMER, J. A., RULE, A. M. & TRIST, M. E., 1920, *Amer. J. Syph.*, **4**, 641.
- KYRLE, J., 1920, *Wien. klin. Wschr.*, **33**, 283 and 313.
- LANGE, C., 1912, *Berl. klin. Wschr.*, **49**, 897.
- LEVADITI, C. & MARIE, A., 1919, *Ann. Inst. Pasteur*, **33**, 741.
- LEVADITI, C., MARIE, A. & NICOLAU, S., 1921, *C.R. Acad. Sci.*, Paris, **172**, 1542.
- MEINICKE, E., 1918, *Berl. klin. Wschr.*, **55**, 83; 1919, *Z. Immunforsch.*, **28**, 280; 1920, *ibid.*, **29**, 396; 1929¹, *Münch. med. Wschr.*, **76**, 318, 628; 1929², *Klin. Wschr.*, **8**, 112, 1080.
- MELKERSON, E., 1928, *Acta med. scand.*, **69**, 119.
- MELLANBY, J. & DAVIES, T. A., 1923, *Brit. J. Exp. Path.*, **4**, 132.
- MILLER, S. R., BRUSH, N. D., HAMMERS, J. S. & FELTON, LL. D., 1915, *Johns Hopk. Hosp. Bull.*, **26**, 391.
- MOORE, J. E., 1921, *Johns Hopk. Hosp. Bull.*, **33**, 231; 1928, *Arch. Derm. Syph.*, N.Y., **18**, 99.
- MOTT, F. W., 1916, *Report of the Royal Commission on Venereal Diseases*, p. 143.
- MÜLLER, R., 1925, *Deuts. Dermatol. Kongr.*, cited by MÜLLER, 1928, *Deuts. med. Wschr.*, **54**, 1032; 1928, *Rep. League Nations Hlth. Org. Second Laboratory Conference*, p. 133; 1929, *Deuts. med. Wschr.*, **55**, 1624; 1930, *Proc. VIII Int. Cong. Derm. Syph.*, Aug. 5-9.
- MURATA TEST, 1928, *League Nations Hlth. Org. Report of the Second Laboratory Conference on the Sero-diagnosis of Syphilis*, Copenhagen, May-June, p. 137.
- NANU-MUSCEL, J., ALEXANDRESCU, C. & FRIEDMAN, L., 1914, *Münch. med. Wschr.*, **61**, 1271.
- NICHOLS, H. J. & WALKER, J. E., 1923, *J. Exp. Med.*, **37**, 525.
- NICOLE, J. E., 1928, *J. Neurol. Psychopath.*, **9**, 33.
- NICOLE, J. E. & STEEL, J. P., 1925, *Lancet*, Lond., ii, 917.
- NOGUCHI, H., 1911, *J. Exp. Med.*, **14**, 557; 1914, *N.Y. Med. J.*, **100**, 349; 1922, *J. Exp. Med.*, **35**, 391.
- NOGUCHI, H. & BRONFENBRENNER, J., 1911, *J. Exp. Med.*, **13**, 43.
- NONNE, M. & APELT, F., 1908, *Arch. Psychiat. NervenKr.*, **43**, 433.
- O'LEARY, P. & BRUNSTING, L. A., 1930, *J. Amer. Med. Ass.*, **94**, 452.
- PÁNDY, K., 1910, *Neurol. Zbl.*, **27**, 915.
- PINEDA, E. V. & ROXAS-PINEDA, E., 1926, *Philipp. J. Sci.*, **30**, 39.
- PÓVOA, H., 1929, *Folka Med.*, **10**, 279.
- RENAUX, E., 1924, *League Nations Hlth. Org. Investigations on the Sero-diagnosis of Syphilis. Report of the Technical Laboratory Conference*, Copenhagen, Nov.-Dec. 23, C5, M5, III (C.H.148), p. 169.
- ROBINSON, D. O., 1912, *J. Cutan. Dis.*, **30**, 410.
- ROSS, G. W. & JONES, E., 1909, *Brit. Med. J.*, i, 1111.
- RUEDIGER, E. H., 1919, *J. Infect. Dis.*, **24**, 204.
- SACHS, H., 1911, *Berl. klin. Wschr.*, **48**, 2066; 1921, *ibid.*, **58**, 1075.
- SACHS, H. & ALTMANN, K., 1908, *Berl. klin. Wschr.*, **45**, 699.
- SACHS, H. & GEORGI, W., 1918, *Med. klinik.*, **14**, 805.
- SACHS, H. & WITEBSKY, E., 1928¹, *Klin. Wschr.*, **7**, 1233; 1928², *League Nations Hlth. Org. Report of the Second Laboratory Conference*, p. 145.
- SAITO, T., 1929, *Z. Hyg. InfektKr.*, **110**, 603.
- SAUNDERS, H. C. & SPIEGEL, L., 1928, *J. Amer. Med. Ass.*, **91**, 630.
- SCHARFELTER, K., 1928, *Wien. klin. Wschr.*, **41**, 1118.
- SCHNITZER, R., 1929, *Deuts. med. Wschr.*, **55**, 228.
- SCHÜFFNER, 1912, *Z. Hyg. InfektKr.*, **72**, 362.

- SHERRICK, J. W., 1915, *J. Amer. Med. Ass.*, **65**, 404.
 SOLOMON, H. C. & KLAUDER, J. V., 1920, *Arch. Derm. Syph.*, Chicago, **2**, 679.
 SPLAINE, R. D. & CHEEVER, A. W., 1928, *New Engl. J. Med.*, **199**, 971.
 STERN, M. & FRANK, 1930, *Z. ImmunForsch.*, **65**, 276.
 STILLIANS, A. W., 1917, *Interst. Med. J.*, **24**, 589.
 STOKES, J. H., 1917, *J. Amer. Med. Ass.*, **68**, 1092.
 STRICKLER, A., 1921, *Arch. Derm. Syph.*, Chicago, n.s., **3**, 177.
 TANIGUCHI, T., 1920, *J. Path. Bact.*, **23**, 368.
 THOMSEN, O. & BJARNHJEDINSON, S., 1910, *Z. ImmunForsch.*, Tl. I, Orig., **7**, 414.
 THOMSON, D. & MILLS, C. H., 1919, *Lancet*, Lond., **i**, 782.
 TULLOCH, W. J., 1928, *R.A.M.C. Jl.*, **50**, 448.
 UNTERSTEINER, R., 1925, *Klin. Wschr.*, **4**, 266.
 VERNES, A., 1920, *Atlas de Syphilimétrie*, Paris, F. Alcan; 1922, *Trav. Inst. Prophylactique*, Fasc. 1; 1922, *ibid.*, Fasc. 2; 1924, *ibid.*, Fasc. 3; 1926, *ibid.*, Fasc. 5.
 For a clear account of all Vernes's methods of diagnosis, with technical details, employed at the Institut Prophylactique see also LEGER, M. & MARTIN, G., *Ce qu'il faut savoir de la Méthode Syphilimétrique Vernes et des applications du Photomètre*, Paris, N. Maloine & Institut Prophylactique
 WARTHIN, A. S., 1928, *Amer. J. Syph.*, **12**, 301; 1929¹, *Brit. Med. J.*, ii, 236; 1929², *Brit. J. Ven. Dis.*, **5**, 255.
 WARWICK, M., 1921, *Arch. Intern. Med.*, **26**, 238.
 WARWICK, M. & NIXON, C. E., 1920, *Arch. Intern. Med.*, **25**, 119.
 WASSERMANN, H., 1924, *Arch. Intern. Med.*, **29**, 401.
 WATSON, H. F., 1925-6, *Quart. J. Med.*, **19**, 431.
 WHITE, C. F. & BARBER, H., 1919, *Brit. J. Derm. Syph.*, **31**, 5.
 WILLIAMS, J. W., 1920, *Johns. Hopk. Hosp. Bull.*, **31**, 335.
 WITH, C., 1917, *Brain*, **40**, 403.
 WYLER, E. J., 1927, *Brit. J. Ven. Dis.*, **3**, 320; 1929, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. **129**, 8.
 ZALOZIECKI, A., 1909, *Msch. Psychiat. Nervenheilk.*, **26**, 196.

Treatment.

CHEMOTHERAPY.

Mercury.

Mercury used empirically in the treatment of syphilis for over four centuries has been found experimentally to have a low spirochæticidal effect relatively to its toxic effects on the tissues, and is rapidly becoming supplanted by bismuth.

Arsenicals.

The scientific chemotherapy of syphilis owes its origin to (a) the views of Schaudinn that *S. pallida* is biologically related to the trypanosomes; (b) the work of Thomas (1905) on the effect of atoxyl (the sodium salt of paraaminophenylarsenic acid) on trypanosomes; (c) the work of Uhlenhuth and Gross (1907) on the effect of atoxyl on spirillosis of hens; (d) the determination by Ehrlich and Bertheim (1907) of the true chemical constitution of atoxyl. This led Ehrlich with his colleagues to investigate the trypanocidal and spirochæticidal effects of a large number of organic compounds of arsenic, with the result that Ehrlich and Hata (1910) showed the trivalent compound dioxidyaminoarsenobenzene dihydrochloride ('606' or salvarsan) to have a far higher spirochæticidal effect relatively to its toxic effects on the tissues than have such pentavalent compounds as atoxyl and arsaceticin (the sodium salt of acetylparaaminophenylarsenic acid). Both atoxyl and arsaceticin had

been used extensively in the treatment of syphilis, but were acquiring an evil reputation for causing optic neuritis when Ehrlich's '606' was introduced. Salvarsan was at first given intramuscularly, being converted before administration into either the mono- or the disodium compound, but it caused very severe pain and local necrosis, and the intravenous route was substituted. For intravenous administration salvarsan must be converted to the disodium compound, by addition of sodium hydroxide after solution of the powder in water, and to obviate the trouble of this operation the disodium compound was issued at a later date in the dry form under the name of sodium-salvarsan, a preparation which has not been used greatly in this country.

Certain inconveniences attending the use of salvarsan led Ehrlich (1912) to prepare dioxydiaminoarsenobenzene monomethylene sulphonylate of soda, known originally as '914' or as neosalvarsan, and now sold under a variety of trade names but recognized officially in this country as 'Novarsenobenzene'. This preparation is now the most commonly used in clinical practice.

Ehrlich and his colleagues also tried the effect of introducing another metal into the combination, and copper, gold, and platinum salvarsans were produced but were found to be too toxic in relation to their therapeutic effects. Another combination by Karrer, viz. silver salvarsan, the properties of which were investigated after Ehrlich's death by Kolle (1918) and colleagues has, however, proved satisfactory, and silver-salvarsan and neo-silver-salvarsan are now used extensively in clinical work. Other preparations which have gained a footing are dioxydiaminoarsenobenzene formaldehyde bisulphite of soda known officially in this country as 'sulpharsenobenzene'; the solution of the base ('592') in glucose which is sold as stabilarsan; and sulfoxyl-salvarsan.

Sulpharsenobenzene is more stable than '914', less active when given intravenously, but more suitable for intramuscular injection. Sulfoxyl-salvarsan is intended for so-called Wassermann-fast cases. It is said to be more active against the spirochætes of recurrent fever than is '914'.

In more recent years certain pentavalent compounds have come into use such as 3-acetylamino-4-hydroxyphenylarsinic acid, or stovarsol, introduced by Levaditi and Navarro-Martin, Fournier, Guénot and Schwartz (1922) for oral administration in the prevention of syphilis, and particularly tryparsamide or the sodium salt of N-phenylglycinamido-p-arsonic acid prepared by Jacobs and Heidelberger (1919), first reported on by Brown and Pearce (1919) of the Rockefeller Institute and now used extensively in the treatment of parenchymatous neurosyphilis.

An important fact must be remembered in both clinical and experimental work, viz. that the arsenobenzene preparations do not act as such on the spirochætes but by virtue of a derivative, an arsenoxide, formed by the tissues, and that the activity of the tissues in this respect apparently varies. I have experience of early cases of syphilis who received only a single dose of '606' and when examined many years later showed no

sign of disease, whether serological or clinical, and of other cases in the same early stages who have remained serologically resistant in spite of many injections. A good example of such refractoriness was published by Hoffmann and Armuzzi (1927). A girl, after relapsing with signs of secondary syphilis, was found to be so unresponsive to treatment that *S. pallida* was demonstrated in her lesions after she had received 4·12 gm. sodium-salvarsan. After two treatments by Zittmann's sweating method, each lasting two days, she responded to the arsenical injections and rapidly became negative. Rabbits were infected with secretion from the lesions after this patient had received six injections of sodium-salvarsan. These rabbits responded to treatment just as well as rabbits infected with other strains of *S. pallida*.

The question of arsenic-fastness in S. pallida.

The ease with which trypanosomes become resistant to chemotherapeutic preparations suggests the possibility of *S. pallida* also becoming resistant if subjected to repeated small doses of arsenobenzene compounds. So far, however, as can be inferred from experiments on animals, the evidence appears to be negative. Margulies (1910) did not produce arsenobenzene-fast strains in rabbit syphilis, though the commencing doses were only a tenth to a fifteenth of the curative. Unfortunately in these experiments the lymph glands of the rabbits supposed to have been sterilized after preliminary subcurative treatment were not tested for infectivity. Akatsu and Noguchi (1917) accustomed cultural *S. pallida* to grow in media containing eventually five and a half times as much salvarsan as they would tolerate at first. Klauder (1924) treated syphilitic rabbits with subcurative doses of salvarsan and later inoculated other rabbits with their glands. After six passages on these lines he found that the sterilizing dose of the '606' had been increased by 68 per cent. Voegtlin (1929) found that syphilitic rabbits were sterilized by four doses of 0·6 mgm. '606' per kgm., i.e. a quarter the single sterilizing dose, administered at intervals of six days. Oelze (1928), after reviewing the literature on the subject, concluded that no experimental proof of salvarsan resistance had been afforded; Klauder's results he regarded as being within the limits of possible error. He added that in cases such as that of Hoffmann and Armuzzi (mentioned above) and of Krantz, Navarro, Nothaas, Frei and Jessner respectively, the fact that the spirochætes were not resistant after transfer to rabbits showed that one should not speak of salvarsan-resistant spirochætes but of salvarsan-inert patients. With this view Moore and Robinson (1930) substantially agree.

Standardization of arsenobenzene preparations.

Although theoretically the arsenobenzene compounds are known chemical entities, successive batches of which should be uniform in toxicity, they may vary considerably in this respect, and it has therefore been customary to submit every batch to a test of toxicity. The subject is, however, one of considerable complexity.

Durham, Gaddum and Marchal (1929) found, for instance, that small mice were more susceptible than large mice, weight for weight, to novarsenobenzene. They also found that therapeutic efficiency is not necessarily proportional to toxicity. Clinical observations, likewise, with different brands of '914' suggested that some preparations which were acceptable by reason of their low toxicity might therapeutically be less efficient than was desirable. Resulting investigations on this drug by Dale and White (1922) showed the suspicion to be well-founded both in regard to the trypanocidal effects in animals and to spirochæticidal in the case of human beings.

In consequence of these and other facts, international conferences have formulated standards and methods of testing toxicity and efficiency of these drugs (see M.R.C. Special Reports Nos. 44 and 128; International Conferences on Biological Standards, Health Organization, League of Nations, 1925 and 1928; Therapeutic Substances Regulations, 1927).

Bismuth.

Following on the work of Robert and Saùton (1916), who had had encouraging results in the prevention and cure of fowl spirochætosis with bismuth salts, Sazerac and Levaditi (1921^{1 & 2}) found that tartro-bismuthate of potassium and sodium exercised a curative effect on rabbit syphilis. The results were first confirmed on man by Fournier and Guénot (1922), and since then a large literature has accumulated which goes to show that bismuth is a very efficient substitute for mercury in the treatment of syphilis when injected intramuscularly; intravenously, it is much more toxic and fleeting in therapeutic action. Most workers maintain that bismuth is far superior to mercury, some even place it higher than the arsenobenzene preparations, others are not sure that it is therapeutically superior to mercury, but practically all are agreed that, for treatment by injection, it is preferable to mercury because it is more tolerable.

Levaditi (1924) described experiments to show that bismuth compounds have a feeble action on spirochætes *in vitro* unless the salts have previously been in contact with cellular extracts, of which liver and kidney are the best for the purpose. A combination of an aqueous tissue extract and a bismuth compound results in a precipitate which contains all the spirochæticidal properties of the mixture. Levaditi calls the active toxalbumin compound formed by contact with cells, whether *in vitro* or *in vivo*—'bismoxyl'. With an increasing content of bismuth, bismoxyl becomes more active up to an optimum; after this any further addition of bismuth is without effect.

In a later paper Levaditi, Nicolau, Schoen, Girard and Manin (1926) showed that after injection of bismuth compounds the bismuth content of syphilomata such as chancres undergoing resolution was infinitesimal (between 4 and 10 thousandths of a milligramme per gramme), and concluded that the metal acted as a catalyser to the spirochæticidal substances elaborated by the tissues. Further work by Levaditi, Sanchis-Bayarri,

Schoen and Manin published in 1927 and 1928 went to show that the prophylactic and curative properties of bismuth are exercised only so long as the tissues, other than those at the site of injection, contain not less than a certain minimal amount of the metal. Injection of the maximum tolerated dose of a soluble preparation of iodo-bismuthate of quinine protected against subsequent inoculation for less than 31 days, while the insoluble basic tartrate, which was excreted much more slowly, protected for as long as 152 days. Bismuth may prevent chancre formation, as shown by Kolle and Evers (1926) in rabbits, so long as the depot of bismuth remains, although spirochætes spread to the lymph glands. When the depot is excised, even two or three months after inoculation, the chancre develops.

Compounds of Bismuth and Arsenic.

Various combinations of bismuth with arsenical compounds were first tried by Ehrlich and Karrer. Stokes and Chambers (1927) reported fairly favourably from the clinical standpoint on bismuth arspenamine sulphonate (Bismarsen) prepared by Raiziss. Levaditi (1925), by combining the sodium salt of p-hydroxy-m-acetylaminophenylarsinic acid (sodium stovarsol) with sodium potassium bismuth tartrate, produced a basic bismuth salt of p-hydroxy-m-acetylaminophenylarsinic acid, which was called 'bistovol'. This compound was found to be active in experimental syphilis, and its effects on human syphilis were reported on favourably by Fournier and Schwartz (1925). Later experimental work by Levaditi (1928) confirmed him in the view that bistovol, whether administered in solid form by mouth or intramuscularly in solution, is an active and safe antisypilitic remedy. Levaditi investigated bismuth arsanilate first prepared by Shircore (1926) and found that in syphilis of animals it had the same properties as bistovol. These results were supported in the case of human syphilis by Fournier and Guénot. Bismuth arsanilate had the advantage over bistovol of being painless when suspended in oil. Bismuth tryparsamide was not found to be so active as the other two.

The Salts of Other Metals.

Besides arsenic, mercury and bismuth, Levaditi (1927) has investigated 27 other elements for spirochæticidal properties and found that, of these, only vanadium, tellurium, platinum and gold are active; also that the spirochæticidal activity of a metal is not related to its atomic weight or to its place in Mendeléeff's system. Thus thallium and lead with atomic weights of 204 and 207 are inactive, while mercury and bismuth with atomic weights of 200 and 209 are active. Tellurium and selenium are very similar chemically and belong to the same group in the Mendeléeff system, but selenium is inactive.

PYROGENETIC THERAPY.

The beneficial effect of an intercurrent infection or a fever on general paresis has been recognized by clinicians for a very long time (see

Gerstmann, 1928¹). Hippocrates, Galen, Boerhaave and Sydenham each noticed that the insane may improve after an acute fever, and numerous writers in the nineteenth century observed long remissions following typhoid, typhus, smallpox, and a large variety of other fevers, as well as after acute suppurations. Rosenblum (1874-5), probably in collaboration with Motschutkoffsky, practised inoculation with relapsing fever on some cases of 'chronic psychosis', with good effects, and in 1854 Jakobi introduced, and L. Mayer continued, a treatment of general paresis by rubbing into the scalp tartar emetic ointment followed by ung. basilicum. Since 1887 Wagner-Jauregg and his colleagues in Vienna have systematically investigated the effects on general paresis of fever produced by various means, including injection of such substances as milk, egg-albumin and sodium nucleinate, of tuberculin and bacterial vaccines and the induction of relapsing and malarial fevers. Of all these, the best was found by Wagner-Jauregg (1918-19) to be infection with malarial fever (*P. vivax*), and this form of treatment has now been employed in many thousands of cases throughout the world.

Treatment with Malarial Fever.

The results reported by various observers have shown that, of the total cases of general paresis, early, medium and late, treated with malarial fever, approximately 30 per cent. have been discharged in full remission, while about the same proportion have shown considerable improvement. Meagher's (1929) admirable report on 1,597 cases of general paresis treated in mental hospitals in England and Wales from July, 1922, to July, 1927, may be cited as an example. It showed that by January, 1928, 33·9 per cent. had died; 40·8 per cent. were still in hospital; and 25·3 per cent. had been discharged. Of the 404 discharged cases, 321 were following an occupation, and 41 were mentally fit but not working, owing to physical or other causes. Of the 652 still in hospital, 238 showed improvement. The percentage of full remissions in mental hospital cases cannot be expected to be so high as in others because the hospital cases are, on the whole, late ones, and experience shows that the best results are obtained in the earlier stages. Driver, Gammel and Karnosh's (1926) collection of 2,336 cases treated in various clinics showed 27·5 per cent. full remissions and 26·5 per cent. incomplete, while Gerstmann's (1928²) report on 400 cases treated in Wagner-Jauregg's clinic before the end of 1922 showed 32 per cent. full remissions without relapse in a period of observation extending from five to over ten years. In a further 12·8 per cent. the disease had been arrested.

The beneficial effects of fever therapy in general paresis have led to its extension to the treatment of tabes and other forms of neurosyphilis, as also to latent cases in which prolonged antisyphilitic treatment has failed to convert the serum or/and spinal fluid reactions to negative. Later Kyrle (1917) reported better results in secondary and later cases from combined fever and salvarsan treatment than from salvarsan alone.

With regard to the results in latent cases, Finger (1928) reported that in 127 treated with large amounts of neosalvarsan in conjunction with mercury and bismuth, only 9 had become serologically negative. The remaining 118 were then treated by his salvarsan-malaria-salvarsan method and 72 became completely negative. According to Finger, malarial treatment acts more particularly on syphilis of the central nervous system than on that of other parts of the body, and Bering (1928) contrasted the indifferent results obtained by ordinary specific treatment in cases with positive spinal fluid as compared with those following malaria plus specific treatment. Gerstmann (1928³) quotes a number of examples of the effect of fever in causing disappearance of *S. pallida* from secondary lesions and from the brain (1928⁴). On the other hand, in cases where the treatment has failed or been only partially successful, spirochaetes have been found in the brain, either after Neisser-Pollak puncture or *post mortem*.

Histological changes have varied according to the relation of the death to the date of the treatment. In some cases in which death occurred in the early stages of the treatment changes indicating an exacerbation of the process have been recorded ; this accords with the increase of symptoms often noted in patients during the period of the fever paroxysms. In cases where the examination was carried out long after the cure and in which the result had been good, the histological changes showed a corresponding degree of recovery.

The serum and fluid changes are said not to be parallel with the clinical, but it would perhaps be truer to say that this is chiefly applicable to the period immediately following the treatment. As the interval lengthens the proportion of cases showing reversion of the fluid and serum changes towards normal increases, a fact which lends support to the theory that the Wassermann and flocculation reactions depend on tissue changes. Gerstmann mentions that in Wagner-Jauregg's clinic it is the rule to repeat the malarial treatment if the reactions remain definitely positive for a year after the first course. In such cases the second course is followed by improvement of the fluid sooner than of the serum.

The Use of Other Pyrogenetic Agents.

Plaut and Steiner (1919-20) recommended infection with relapsing fever, using *S. duttoni* on the grounds of its possibly provoking the formation of antibody which would extend to *S. pallida*. Other workers, like Wagner-Jauregg at first, have employed such agents as T.A.B. vaccine, and Schroeder (1928) has claimed good results from injection of a sulphur oil (Sulfosin) which produces a rise of temperature to 104° F. or more. The general view regarding other agencies is that they do not afford such good results as malaria, though they are more controllable and safer.

Mode of Action.

Opinions are divided as to the mode of action of pyrogenetic therapy. A plausible explanation is the susceptibility of *S. pallida* to temperature.

Weichbrodt and Jahnel (1919) raised the body temperatures of rabbits with chancres by putting them into an incubator at 41° C. for half an hour twice daily. The chancres healed in three to five weeks, instead of persisting for months as is customary in untreated rabbits. Schamberg and Rule (1926, 1927 and 1928) found that by immersing rabbits in a hot bath at 113° F. for 15 to 20 minutes daily and so raising their temperatures to 105·4 to 110° C. they could prevent the development of syphilis after inoculation, or promote the rapid healing of chancres after these had appeared. In the prophylactic experiments, of 9 rabbits inoculated and then treated with 9, 10 or 11 baths, none developed syphilis. Of 10 other inoculated rabbits treated with from 1 to 7 baths, 1 died prematurely, 4 became infected and 5 escaped infection. In the therapeutic experiment 2 rabbits with primary syphilomata were treated with 12 and 15 baths respectively. In both *S. pallida* was found abundantly after the third bath (maxima so far, 106° F. and 107° F.) but not after the eighth (maxima so far, 107·3° F. and 109° F.). The chancres had practically healed by this time. Inoculation of other animals with gland material from these rabbits 75 days after infection gave negative results. In a rabbit with a secondary syphiloma similar treatment failed to sterilize.

Experiments on the same lines with patients whose temperatures were raised to 105° F. or 106° F., reported by Schamberg and Tseng (1927), were inconclusive, but the authors' suggestion that hot baths could be a useful adjuvant to specific treatment accords with the statement of Finger (1928) that Kyrle found injections of milk (which raised the temperature to 104° F.) in conjunction with mercury salicylate acted better than did mercury salicylate alone. Frazier (1927) repeated Schamberg and Rule's experiments and found generally that to produce a curative effect the body temperature must be raised to between 106·2° F. and 110·6° F.

Bessemans, de Potter and Hacquaert (1928), independently of Schamberg and Rule, tried the effect of immersion of rabbits with primary syphilomata in hot water. They found that if the interior of the syphiloma could be kept at 40° C. for 2 hours, or at 42° C. for 1 hour, the spirochætes became immobilized, and the lesions healed quickly. Bessemans, Vercoullie and Hacquaert (1929) have reported good results in the treatment of chancres in two men by directing on the lesions a stream of hot air at about 44° C. for an hour at a time.

In a later paper Bessemans (1929) reviewed previous experimental work on the treatment of syphilis with hot baths and presented further arguments in favour of the theory that the temperature is an important factor in the curative effect. He pointed out that in his experiments and those of Schamberg and colleagues only external lesions which were exposed to the full effects of the physical heat were influenced. In rabbits which were inoculated and the inoculated testicles immersed in hot water so that the intratesticular temperature remained at 39° C. for 6 hours, syphilitic lesions developed in normal time, whereas, if the water was

hot enough to raise the intratesticular temperature to 42° C. for 1 hour or to 40° C. for 2 hours, no local lesion developed, though the application was made as long as 15 days after inoculation. Bessemans thought that *S. pallida* has a predilection for tissues at a comparatively low temperature, e.g. in a rabbit the cornea and the descended testicle. He related experiments in his laboratory by Hacquaert in which both testicles were inoculated and one of them was pushed back into the abdominal cavity. This developed no lesion, and examination showed no *S. pallida* in it. In another case the inoculated testicle was left in the abdominal cavity for only one day. On being replaced motile *S. pallida* were found in it, but three days later they had disappeared, and in ten days the testicle was normal. A testicle with a syphiloma rich in *S. pallida* was pushed back into the abdominal cavity and at the end of three hours was replaced in the scrotum. No *S. pallida* was found in it, and the syphiloma healed rapidly. Thus, an organ which is normally very susceptible to *S. pallida* was quite otherwise when placed in conditions exposing it to higher temperatures. Accordingly, although admitting that other factors play a part in the curative effect of fever, Bessemans regards heat as an important one.

The probability that heat is not the only factor is suggested by the fact that good effects have been experienced in cases where the temperature did not reach 104° F. The theory has been advanced that the pyrogenetic agent stimulates an increase in antibodies generally and that these act also against *S. pallida*. As mentioned, this is the explanation offered by Plaut and Steiner. Busson (1927) found that in the crisis of a malarial attack the red blood-cells are strongly toxic for guinea-pigs, unlike the cells of a patient whose temperature has been raised by an injection of saprovan, and sees in this breaking down of the blood-cells a powerful protein shock therapy. Schilling, Jossmann, Hoffmann, Rubitschung and van der Spek (1924) see in malarial therapy an ideal protein shock therapy which in each paroxysm stimulates an increase in turn of attacking neutrophils, of defensive monocytes and of healing lymphocytes and eosinophils, which is followed by a great regeneration of red blood-cells.

On the question of antibody production by malarial therapy may be mentioned some recent work by Caldwell (1930) in which the serum of general paretics treated with malaria not more than five months previously inhibited the growth of cultural *S. pallida* (Noguchi's and Zinsser's strains). On the other hand, the spirochæticidal effect of sera taken at longer intervals after the treatment appeared to have declined until after eighteen months there was no difference between the serum of malaria-treated cases and that of the untreated. Hoff and Silberstein (1925) found that a rapid rise in serum antibodies against various bacteria (staphylococci, streptococci and *B. coli*) occurred early in the first paroxysm of malaria and that this extended in the third paroxysm to the spinal fluid. Further, a combination of the serum, or the spinal fluid, and leucocytes (obtained by injection of turpentine) immobilized and destroyed

S. pallida, as observed microscopically, and when mixed with particles of syphilitic testicle prevented infection. In contrast with this, the fluid of untreated cases was without effect. In a later article (1926) they related similar experiments with patients treated with relapsing fever; the effects were not so marked as in malaria-treated cases.

On the possibility of a direct antagonism between the fever-producing organism and *S. pallida* Kritschewski (1929) may be cited. He referred to the excellent effect of relapsing fever on general paresis and tabes reported by Plaut and Steiner and others, and carried out some experiments to discover if it was due to antagonism between *S. duttoni* and *S. pallida*, or alternatively to production of antibodies to *S. duttoni* which extended to *S. pallida*. He infected 69 mice with syphilis, and 38 days later with a strain of *S. duttoni*, which invades the brain in 78.6 per cent. of cases. After three attacks of fever, brain material of the mice was inoculated into rabbits' testicles, which became infected in the same proportion as did rabbits inoculated with brain of syphilized mice not treated with *S. duttoni*. These experiments support those of Schlossberger and Prigge (1926), who infected rabbits suffering from yaws and from syphilis with relapsing fever. No rise of temperature occurred and no effect on the syphilitic or frambœsial infection followed. Schmidt-Ott (1927) infected syphilitic rabbits with trypanosomes and later treated them with sub-curative doses of Bayer-205. *S. pallida* disappeared quickly from the lesions, which healed quickly, but the infection was not eradicated. The possible explanation of the therapeutic effect was the action on *S. pallida* of disintegration products of killed trypanosomes, but equally the explanation might lie in a stimulation of the defensive mechanism by non-specific proteins.

Vascular dilatation produced by the fever and hence an increased accessibility of *S. pallida* to attack by antibodies, or by specific remedies, has been advanced as an explanation by a number of workers. In this connection may be quoted some observations by Pamboukis (1929), in which a case of general paresis with right hemiplegia of a month's duration, a case of Parkinson's disease and one of hemiplegia with motor aphasia, and two cases of coronary sclerosis with frequent paroxysms of angina pectoris cleared up or improved considerably under an intercurrent attack of dengue. Dengue is characterized by leucopenia, which seems to controvert the theory that fever acts by provoking a leucocytosis. On the other hand, it causes a wide dilatation of blood-vessels. The fact that most workers now agree on the value, even the necessity, of combining fever-treatment with injections of arsenobenzene preparations and other specific remedies seems to imply a belief in the theory that fever makes the spirochætes more accessible.

Wagner-Jauregg (1925) supposes that malarial treatment, besides acting on the luetic process generally, has a selective effect on the disease in the parenchyma of the nervous system, and advances arguments in support of this hypothesis, such as (a) the slowness of the effect of malarial

treatment on vascular syphilis, (b) the strong cerebral reaction during the malarial treatment, and (c) the difference in the response of the serum reactions from that of the fluid, the former being more resistant.

The occasional development of gummata in malaria-treated cases has suggested to some workers that fever-treatment stimulates the tissues outside the nervous system to react against *S. pallida* and so to form protective substances. Goeckermann (1928) reported the case of a paretic who five months after a malarial cure developed a nodular cutaneous syphilide on one thigh, suggesting that the malaria had made the skin allergic, with consequent increase of the defence forces. Goeckermann cited as other evidence that the therapeutic effect of fever is due to action on the tissues rather than on spirochaetes the slowness of improvement in the spinal fluid reactions. Gerstmann seems to favour the same hypothesis, and cites observations in support of it. He calls attention to the observation by Skalweit (1925) that in ordinary cerebral syphilis the blood-picture is that of a lymphocytosis while in a paretic before malarial treatment there is a lymphopenia, but after malarial treatment cases which do well show a strong lymphocytosis.

Such experimental evidence as is available, therefore, seems to show that, in the curative effect of fever treatment, there are a number of factors of which the lethal, or at any rate the damaging, effect of heat on *S. pallida* is perhaps the most important. The superior effect of malaria, although other agents can produce rises of temperature as high as those in malarial fever, seems to indicate an antigenic effect of this type of infection which is greater than that of others, but whether this is by virtue of the parasites, of broken-up red corpuscles, or of other products resulting from malarial fever, remains undecided. Excellent results have been recorded after purely fever treatment, but on the whole better when this has been supplemented by injections of salvarsan and other specific remedies.

In syphilis the balance tends usually to the side of the tissues, as is testified by the long periods of latency. This may be broken by lowering of the tissue resistance as a result of injury, but can often then be restored without the help of any specific remedy. For example, in the history of a nodular cutaneous syphilide one may find that former outbreaks have healed by poulticing; tabes often enough becomes arrested without treatment, and in general paresis spontaneous remissions occur. Considering these facts it is perhaps not surprising that any of a number of factors lowering the vitality of *S. pallida* on the one hand or raising the tissue resistance on the other, can be responsible for terminating the activity of a syphilitic process.

REFERENCES.

- AKATSU, S. & NOGUCHI, H., 1917, *J. Exp. Med.*, **25**, 349.
 BERING, F., 1928, *Wien. klin. Wschr.*, **41**, 977.
 BESSEMANS, A., 1929, *Brux. méd.*, **9**, 1130.
 BESSEMANS, A., DE POTTER, F. & HACQUAERT, R., 1928, *C.R. Soc. Biol.*, Paris, **99**, 1610; 1929, *ibid.*, **101**, 163.
 BESSEMANS, A., VERCOULLIE, J. & HACQUAERT, R., 1929, *Rev. belge Sci. méd.*, **1**, 425.

- BROWN, W. H. & PEARCE, L., 1919, *J. Exp. Med.*, **30**, 417.
- BUSSON, B., 1927, *Wien. klin. Wschr.*, **40**, 577.
- CALDWELL, W. A., 1930, *Brit. J. Exp. Path.*, **11**, 1.
- DALE, H. H. & WHITE, C. F., 1922, *Lancet*, Lond., i, 779.
- DRIVER, J. R., GAMMEL, J. A. & KARNOSH, L. J., 1926, *J. Amer. Med. Ass.*, **87**, 1821.
- DURHAM, F. M., GADDUM, J. H. & MARCHAL, J. E., 1929, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 128.
- EHRlich, P., 1912, ref. SCHREIBER, E., 1912, *Münch. med. Wschr.*, **59**, 905.
- EHRlich, P. & BERTHEIM, A., 1907, *Med. Klinik.*, **43**, 1298.
- EHRlich, P. & HATA, S., 1910, *Die experimentelle Chemotherapie der Spirillosen*, Berlin (Springer).
- FINGER, E., 1928, *Wien. klin. Wschr.*, **41**, 771.
- FOURNIER, L. & GUÉNOT, L., 1922, *Ann. Inst. Pasteur*, **36**, 14.
- FOURNIER, L. & SCHWARTZ, A., 1925, *C.R. Acad. Sci.*, Paris, **180**, 1973.
- FRAZIER, C. N., 1927, *Arch. Derm. Syph.*, N.Y., **16**, 445.
- GERSTMANN, J., 1928¹, *Die Malariabehandlung der progressiven Paralyse*, 2nd Edn. (Vienna), Springer, p. 5; 1928², *ibid.*, p. 117; 1928³, *ibid.*, p. 207; 1928⁴, *ibid.*, p. 182; 1928⁵, *ibid.*, p. 162; 1928⁶, *ibid.*, p. 216.
- GOECKERMANN, W. H., 1928, *Amer. J. Med. Sci.*, **175**, 261.
- HOFF, H. & SILBERSTEIN, F., 1925, *Z. ges. exp. Med.*, **48**, 6; 1926, *ibid.*, **49**, 294.
- HOFFMANN, E. & ARMUZZI, G., 1927, *Deuts. med. Wschr.*, **53**, 51.
- JACOBS, W. A. & HEIDELBERGER, M., 1919, *J. Exp. Med.*, **30**, 411.
- JAKOBI, 1854, cited by GERSTMANN, p. 7.
- KLAUDER, J. V., 1924, *Arch. Derm. Syph.*, N.Y., **9**, 446.
- KOLLE, W., 1918, *Deuts. med. Wschr.*, **44**, 1177, 1211.
- KOLLE, W. & EVERS, E., 1926, *Deuts. med. Wschr.*, **52**, 557.
- KRITSCHESKI, I. L., 1929, *Klin. Wschr.*, **8**, 1259.
- KYRLE, J., 1917, *Wien. klin. Wschr.*, **30**, 707; 1924, *ibid.*, **37**, 1105.
- LEVADITI, C., 1924, *J. State Med.*, **32**, 62; 1925, *C.R. Acad. Sci.*, Paris, **180**, 1971; 1927, *C.R. Soc. Biol.*, Paris, **97**, 167; 1928, *Brit. J. Ven. Dis.*, **4**, 25.
- LEVADITI, C. & MANIN, Y., 1927, *C.R. Soc. Biol.*, Paris, **97**, 655.
- LEVADITI, C. & NAVARRO-MARTIN, L., FOURNIER, L., GUÉNOT, L. & SCHWARTZ, A., 1922, *Ann. Inst. Pasteur*, **36**, 729.
- LEVADITI, C., NICOLAU, S., SCHOEN, R., GIRARD, M. A. & MANIN, Y., 1926, *Ann. Inst. Pasteur*, **40**, 541.
- LEVADITI, C., SANCHIS-BAYARRI, V. & MANIN, Y., 1927, *C.R. Soc. Biol.*, Paris, **97**, 1687.
- LEVADITI, C., SANCHIS-BAYARRI, V., SCHOEN, R. & MANIN, Y., 1928, *Ann. Inst. Pasteur*, **42**, 105.
- MARGULIES, M., 1910, *Deuts. med. Wschr.*, **36**, 1907.
- MCINTOSH, J., FILDES, P. & DEARDEN, H., 1912, *Z. ImmunForsch.*, Tl. I, Orig., **12**, 164.
- MEAGHER, E. T., 1929, *Board of Control on General Paralysis and its Treatment by Induced Malaria*, London, H.M. Stationery Office.
- MOORE, J. E. & ROBINSON, H. M., 1930, *Arch. Derm. Syph.*, N.Y., **21**, 32.
- MOTSCHUTKOFFSKY, 1876, cited by GERSTMANN, p. 6 (footnote).
- OELZE, F. W., 1928, *Arb. Inst. exp. Ther. Frankfurt*, Nr. **21**, 120.
- PAMBOUKIS, G., 1929, *Deuts. med. Wschr.*, **55**, 484.
- PLAUT, F. & STEINER, G., 1919-20, *Z. ges. Neurol. Psychiat.*, **53**, 103; 1922, *ibid.*, **75**, 686.
- ROBERT, A. E. & SAUTON, B., 1916, *Ann. Inst. Pasteur*, **30**, 231.
- SAZERAC, R. & LEVADITI, C., 1921¹, *C.R. Acad. Sci.*, Paris, **172**, 1391; 1921², *ibid.*, **173**, 338.
- SCHAMBERG, J. F. & RULE, A. M., 1926, *Arch. Derm. Syph.*, Chicago, **14**, 243; 1927, *J. Amer. Med. Ass.*, **88**, 1217; 1928, *Arch. Derm. Syph.*, Chicago, **17**, 322.
- SCHAMBERG, J. F. & TSENG, H., 1927, *Amer. J. Syph.*, **11**, 337.
- SCHILLING, V., JOSSMANN, HOFFMANN, K., RUBITSCHUNG & VAN DER SPEK, 1924, *Z. klin. Med.*, **100**, 742.
- SCHLOSSBERGER, H. & PRIGGE, R., 1926, *Med. klinik.*, **22**, 1227.
- SCHMIDT-OTT, A., 1927, *Münch. med. Wschr.*, **74**, 1488.
- SCHROEDER, K., 1928, *Klin. Wschr.*, **7**, 1636.

- SHIRCORE, J. O., 1926, *Lancet*, Lond., ii, 43.
 SKALWEIT, W., 1925, *Z. klin. Med.*, **102**, 258.
 STOKES, J. H. & CHAMBERS, S. O., 1927, *J. Amer. Med. Ass.*, **89**, 1500.
 THOMAS, H. W., 1905, *Proc. Roy. Soc. B.*, **76**, 589.
 THOMAS, H. W. & BREINL, A., 1905, *Report on Trypanosomes, Trypanosomiasis and Sleeping Sickness Mem. Lpool. Sch. Trop. Med.*, **16**.
 UHLENHUTH, P., 1911, *Experimentelle Grundlagen der Chemotherapie der Spirochaetenkrankheiten*, Berlin & Vienna, Urban & Schwarzenberg.
 UHLENHUTH, P. & GROSS, 1907, *Arb. Gesundheitsamt.*, Berl., **27**, 231.
 VOEGTLIN, C., 1929, *J. Pharmacol.*, **35**, 189.
 WAGNER-JAUREGG, J., 1887, *Jahrb. Psychiat. Neurol.*, **7**, 94; 1918-19, *Psychiat.-neurol. Wschr.*, **20**, 132 & 251; 1922, *J. Nerv. Ment. Dis.*, **55**, 369; 1925, cited by GERSTMANN, p. 212.
 WEICHBRODT, R. & JAHNEL, F., 1919, *Deuts. med. Wschr.*, **45**, 483.

***Spirochaeta cuniculi* Noguchi (1921).**

BY EDWARD HINDLE.

Synonym : *Spirochaeta paraluis cuniculi* Jacobsthal (1920).

This organism was first observed by E. H. Ross (1912) and subsequently described by H. Bayon (1913) from superficial sores in the ano-genital region and also in the neighbourhood of the mouth of rabbits. Subsequently, the infection caused by this spirochaete, known as rabbit syphilis or rabbit spirochaetosis, has been extensively studied, since in some ways it bears a superficial resemblance to human syphilis.

S. cuniculi is generally admitted to be indistinguishable morphologically from *S. pallida* and *S. pertenuis*, consequently it is of some practical importance in experiments with the two latter species in rabbits to be able to distinguish them from *S. cuniculi*.

Pathogenicity.

S. cuniculi produces only a localized infection of the epidermis, in this respect differing markedly from *S. pallida*. The incubation period of the disease varies from 2 to 10 weeks, after which an inflammation develops in the affected region of the skin, generally the perineum, and very rapidly the tissues become swollen and covered with sores. Under the sore is a smooth oedematous surface, and on puncturing this a liquid is obtained in which may be found large numbers of the spirochaete, often agglutinated in tangled masses. In nature the infection generally seems to be transmitted from one animal to another during coitus, though doubtless it may be spread by simple contact. Experimentally, the disease can readily be produced by rubbing infected material containing the spirochaetes on to the scarified epidermis in the region of the perineum, or on the prepuce or scrotum. The eyelids and nose are also susceptible, but it is difficult to produce infection by inoculation in the region of the back.

The spirochaetes produce scrotal lesions when inoculated into the testis, and keratitis when inoculated into the anterior chamber of the eye, following which a generalized infection of the skin has been observed (Klarenbeek, 1923). Although generally localized in the skin, very scanty

spirochætes have been recovered from the lymphatics and blood of infected rabbits (Warthin, Buffington and Wanstrom, 1923), although they have never been found in silver-stained sections of the internal organs.

Up to the present all attempts to infect other mammals, including man, have been uniformly negative, and it would seem that the disease is a specific infection of rabbits.

In view of the close resemblance of *S. cuniculi* and *S. pallida*, cross-immunity experiments have been made with these species in rabbits. Schereschewsky (1920) found that rabbits that had acquired immunity against *S. pallida* could be infected with *S. cuniculi*, and conversely a rabbit infected with *S. cuniculi*, when inoculated with the human virus, developed a typical parenchymatous keratitis. According to Danila and Stroë (1914) the two infections can develop simultaneously and each keep its own characters through several passages. It is doubtful, however, whether cross-immunity experiments are of any significance in this disease, for Klarenbeek (1923) has shown that after recovery from infection with *S. cuniculi*, rabbits can be reinfected with the same strain. Similar results were also obtained by Adams, Cappell and McCluskie (1928).

In view of the widespread occurrence of spontaneous infection with *S. cuniculi* in rabbits, some of the results obtained by the passage of *S. pallida* in these animals are open to question.

Levaditi and Marie (1923) described a neurotropic strain of *S. pallida* which, in marked contrast with other neurotropic strains, produced only superficial lesions in rabbits. Moreover, the spirochætes from these lesions were not pathogenic to man. There is no very definite evidence that passage in rabbits affects pathogenicity, for Pettit (1928) found that after passage in rabbits for 22 months, *S. pallida* was still virulent to man, therefore it seems possible that the above-mentioned strain may have been derived from naturally infected animals.

The histology of the disease has been studied by Levaditi, Marie and Isaïcu (1921), Warthin, Buffington and Wanstrom (1923) and others, who all agree that the infection produced by *S. cuniculi* is a superficial lesion, papillomatous or condylomatous in character and limited to the mucous membranes or skin. The lesions are restricted to the epithelial surface and upper portion of the subepithelial tissues, with marked hyperplasia of the epithelium and papillary layer, where large numbers of spirochætes may be observed. The lesions are essentially condylomata and histologically never suggest a chancre.

Cultivation.

Cultivation of *S. cuniculi* seems to present considerable difficulty, for hitherto no one has succeeded in obtaining a pure culture. Adams, Cappell and McCluskie (1928) made a very large number of experiments with various media, including the Smith-Noguchi medium, but although a considerable amount of multiplication was observed in mixed cultures, they were unable to grow the spirochæte in pure culture.

Treatment.

This infection may readily be cured by local application of mercurial ointment, or by injections of arsenicals, tellurium compounds (Levaditi and Nicolau, 1926), or bismuth compounds (Sazerac and Vours, 1924). In the case of treatment with bismuth, which in doses of 0.02 to 0.03 gm. per kg. causes the spirochætes to disappear in 48 hours, phagocytosis is said to play an important part in their destruction.

REFERENCES.

- ADAMS, D. K., CAPPELL, D. F. & MCCLUSKIE, J. A. W., 1928, *J. Path. Bact.*, **31**, 157.
 ARZT, L. & KERL, W., 1914, *Berl. klin. Wschr.*, **27**, 1053.
 BAYON, H., 1913, *Brit. Med. J.*, ii, 1159.
 DANILA, P. & STROË, A., 1914, *C.R. Soc. Biol.*, Paris, **77**, 167.
 JACOBSTHAL, E., 1920, *Derm. Wschr.*, **71**, 569.
 KLARENBECK, A., 1923, *Ann. Inst. Pasteur*, **37**, 886.
 LERSEY, P. & KUCZYNSKI, M., 1921, *Berl. klin. Wschr.*, **58**, 664.
 LEVADITI, C. & MARIE, A., 1923, *Ann. Inst. Pasteur*, **37**, 189.
 LEVADITI, C., MARIE, A. & ISAÏCU, 1921, *C.R. Soc. Biol.*, Paris, **85**, 51.
 LEVADITI, C. & NICOLAU, S., 1926, *C.R. Soc. Biol.*, Paris, **94**, 827.
 NOGUCHI, H., 1921, *J. Amer. Med. Ass.*, **77**, 2052.
 PETTIT, A., 1928, *Contribution à l'étude des spirochétidés*, Vanves.
 ROSS, E. H., 1912, *Brit. Med. J.*, ii, 1651.
 SAZERAC, R. & VAURS, R., 1925, *C.R. Soc. Biol.*, Paris, **91**, 430.
 SCHERESCHEWSKY, J., 1920, *Berl. klin. Wschr.*, **57**, 1142.
 WARTHIN, A. S., BUFFINGTON, E. & WANSTROM, R. C., 1923, *J. Infect. Dis.*, **32**, 315.
 *WORMS, W., 1930, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **7**, 718.
 *ZUELZER, M., 1925, Supplement to Prowazek and Nöller's *Handb. d. pathogenen Protozoen*, Barth, Leipzig.

* Contain very good bibliographies of the subject.

CHAPTER VIII. RAT-BITE FEVER OR SODOKU.

BY A. ROBERTSON

(LONDON SCHOOL OF HYGIENE AND TROPICAL MEDICINE).

Clinical.

THERE are three cardinal symptoms of rat-bite fever or sodoku in man : (1) recurring inflammation at the site of the original lesion, i.e. the bite of the rat or other animal, with involvement of the local lymphatic system ; (2) fever, sudden in onset and remittent in character ; (3) a rash of a curious purplish shade occurring concurrently with the fever.

1. *The wound.* As a rule the wound caused by the bite heals cleanly and by first intention. If it does not do so then there has been superimposed septic infection which may sometimes be mistaken for, or, on the other hand, may equally serve to hide, true rat-bite fever. Septic involvement renders it much more difficult to demonstrate *Spirillum minus* in the exudate. During the incubation period the ' primary ' lesion is entirely quiescent, but, along with the onset of the fever, there is pain in the scar, which together with the surrounding tissues becomes red, swollen and œdematous. In some cases the process goes on to ulceration, said by Arkin (1920) and Adams (1925) to resemble closely an extragenital chancre. The regional lymphatics are inflamed and the glands are swollen and firm in consistence, but are non-adherent. With each recurrent attack of fever there is a return of the pain and swelling in the wound and of the regional lymphadenitis. These symptoms, however, gradually subside, becoming less severe each time until they disappear, leaving nothing but a somewhat bluish or purplish scar.

2. *The fever.* During the febrile attacks the temperature may reach 104° F. in the more severe cases, while, in those of milder intensity, it may never go higher than 101° F. The onset is abrupt and may be initiated by a rigor, and the actual attack lasts about two or three days, the temperature remaining at the same high level all the time. The fall to normal is just as abrupt and as rapid as the onset. Between the pyrexial periods there is an abatement of all the symptoms and the temperature returns to normal. The number of the febrile attacks varies in each case, commonly there are six or eight, or even more, separated by intervals of from three to seven days. In the majority of cases there is a tendency for the disease gradually to subside, the apyrexial intervals becoming longer and the attacks of fever less frequent, shorter in duration, and not so severe.

3. *The rash.* The rash, which is most characteristic, usually appears with the first attack of fever, but if it should fail to do so will be seen during some subsequent febrile period. It is maculo-papular and is found in slightly raised areas of varying size. There is no associated pain or itch,

and, when the temperature falls again, it fades and disappears, but can sometimes be made to reappear even in the periods of remission if the patient is placed in a hot bath. The most definite feature about the eruption is the colour, which is of a curious deep purplish shade in sharp contrast to the normal tint of the skin.

4. *Other symptoms.* Many other symptoms have been described in addition to the above, but most of them can be disregarded for present purposes as accidental accompaniments of the disease. The most important is pain of varying intensity, commonly quite severe, in the muscles especially of the arms and legs and in the neighbourhood of the joints. This, too, tends to come on with the fever and to abate in the remissions. A degree of anæmia, secondary in character, is quite common, and slight alimentary disturbances such as constipation, diarrhœa or even signs of a more severe gastro-enteritis are not infrequent. These might quite well be expected with any condition where fever was such a predominating symptom.

Towards the end of the disease an urticarial rash may be found; this has been regarded from the point of view of prognosis as a hopeful sign. Sometimes there are symptoms of slight kidney trouble evidenced by casts and albumin in the urine, and headache, giddiness, tinnitus, photophobia, patches of anæsthesia and hyperæsthesia have all been noted as symptoms referable to the nervous system. With regard to the blood the degree of anæmia is proportional to the intensity of the infection and the severity and duration of the disease. During the bouts of fever there is often a leucocytosis (12,000 to 20,000) which gives place during the remissions to a relative lymphocytosis. Towards the end of the infection an eosinophilia, present also during the quiescent phases, has been described (Frugoni, 1912; Walch, 1923, and others). A positive Wassermann reaction is exhibited in about 50 per cent. of cases (Blum and Clement, 1925), and, if syphilis can be definitely excluded, may be a useful aid to diagnosis.

Ætiology.

Historical. Although the disease had been recognized for a long period—early accounts were given in 1840 by Wilcox and by Watson in America—it was not until 1902, following the publication of a paper by Miyake, that definite research was carried out with a view to isolating the ætiological agent. The discovery eventually was made in 1915 by Futaki, Takaki, Taniguchi and Osumi (1916). Previous to this, however, Hata (1912) had found that cure resulted from salvarsan injections.

Curiously enough the *Spirillum* had been seen and reported on much earlier by other workers, although it was not then associated in a causal connection with the disease rat-bite fever. Vandyke Carter (1887) was probably the first to see it in the blood of a rat, *Mus decumanus*, in India and his description of its dimensions, movement and behaviour was wonderfully accurate. For this organism, Carter suggested the bestowal of a name as follows: 'a bacterium belonging to the genus spirochæte

. . . . such as from its small dimensions might be named provisionally *Spirillum minor*'. This name has since been corrected to *Spirillum minus* Carter, 1887.

Wenyon (1906) and Breinl and Kinghorn (1906) named the organism—obtained in both instances from the same source—*Spirochæta muris* and *Spirochæta laverani* respectively. Wenyon was of the opinion that his *S. muris* was identical with the spirochæte found by Borrel (1905) in the cancer growths of mice. Other workers (Calkins and Clowes, 1905; Deetjen, 1906, &c.) also described the organism from the same source, viz. neoplastic growths in mice.

The first mention of the terminal flagella was that of MacNeal (1907). This observer adopted the name *Spirochæta muris* var. *virginiana*, although he considered it to be the same organism as was described by Carter in 1887. The association of the organism with rat-bite fever was proved by Futaki, Takaki, Taniguchi and Osumi (1917), who succeeded in demonstrating it in fluid from an inflamed lymph gland and in the skin where the rash was present. They bestowed the name *Spirochæta morsus muris* on it. Almost at the same time other Japanese workers, Ishiware, Ohtawara and Tamura (1917) were successful in finding the *Spirillum* in guinea-pigs experimentally inoculated from human cases and they also managed to show that the disease was transmissible by the bite of the rat. Row (1917 and 1922) was of the opinion that the Indian rat-bite fever parasite was specifically distinct from the Japanese variety. Parmanand (1923), however, was able to prove that the species in India was possessed of flagella (which Row had failed to demonstrate), and therefore Row's name *Spirochæta petit* must also become a synonym.

That the virus of rat-bite fever might not be a spirochæte but a *Spirillum* was first suggested by Zülzer (1921), and later Robertson (1924) confirmed Zülzer's work and definitely referred the virus to the genus *Spirillum*. At the same time the conclusion was arrived at that the *Spirillum* of rat-bite fever was in no way distinct from the naturally occurring organism previously described (by Wenyon, Lingard, Borrel, &c.) in the blood of rats, mice and other animals, and, therefore, following the law of priority, Vandyke Carter's specific name *S. minus* must become valid.

The Causative Organism.

Morphology. The main distinction between *Spirilla* and the true spirochætes is that the bodies of the former are comparatively rigid, and movement is produced by terminal flagella which are absent in the *Spirochæte*. Both *Spirilla* and *Spirochæte* have their bodies in the shape of a spiral, and, as will be seen later, there is now some doubt about the spiral structure of the rat-bite organism. If this is absent it may be necessary once more to alter the generic name.

To get a proper idea of the structure and movement of this parasite it must be examined under dark-ground illumination. Material for such investigation may be obtained in the case of man from the exudate at the

primary sore, from the juice of lymph glands obtained by puncture, or from serum got by scarifying the skin over the areas of rash. In inoculated animals, especially mice, which are the most convenient for this purpose, the parasites are commonly quite numerous in the peripheral blood, and even more so in the peritoneal fluid.

Owing to the rapidity of the movement, which consists of steady but swift forward motion or sudden darting in any direction, it may be difficult to determine the details of structure. Bright light or exposure to cold has the effect of slowing the rate of movement, but care should be exercised that the individuals studied are not in process of degeneration. The body is rigid. It will bend from the application of outside forces but tends to reassume the original shape as soon as the cause of the bending has been removed. At first the shape was supposed to be a true spiral composed of from one and a half to nine twists, but more recently, Ruys (1926) and McDermott (1928) have stated that the waves or undulations lie all in the one plane. The width of the body is probably between 0.1μ and 0.2μ , and the width of the spiral about 0.7μ . The length varies considerably; short forms with only one and a half turns or waves measure 1.5μ and the longer forms up to some 9μ or 10μ with eight or nine twists or undulations. The pitch of the waves from crest to crest is from 0.8μ to 1μ .

The organism as a rule has flagella at both ends, although in some instances (usually the short forms) they may be present at one end only. In number they vary from 1 to 7, but in this connection it is as well to remember that, when the movement is slowed, they tend to twist round each other and thus several may appear as a single thread. In length the flagella may measure up to 7μ or 8μ . When the *Spirillum* is actively moving, the flagella at the then anterior end trail backwards along the body; this may lead to the impression that flagella are present at one end only.

The flagella are very difficult to demonstrate in stained preparations. Occasionally with Leishman's or Giemsa's stains they may appear quite well defined, but this is the exception. Undoubtedly the best, although even this is far from satisfactory, is Tribondeau's modification of Fontana's silver impregnation method (as recommended by Knowles and Das Gupta, 1928). This method has the advantage that all the materials required are to be found in nearly every hospital. The procedure is as follows:

- (1) Thin films are prepared of the suspected material and are allowed to dry in air. They are then fixed in Ruge's fluid for one minute, i.e. glacial acetic acid 1 c.cm., formalin (40 per cent.) 2 c.cm., and distilled water 100 c.cm.

- (2) The fixative is then drained off the slide and is replaced by methyl alcohol. The process is completed by flaming off the methyl alcohol.

- (3) Next the preparation is mordanted in a freshly prepared solution of tannic acid 5 gm., distilled water 100 c.cm. The slide should be warmed gently until steam rises. After mordanting the slide should be subjected to a very thorough washing in running tap-water.

(4) The silver nitrate solution should now be applied. As before, the slide is flooded and warmed for one minute till steam rises. The silver nitrate solution is prepared by adding liq. ammoniæ drop by drop, shaking all the time, to a 5 per cent. solution of silver nitrate in distilled water until the precipitate redissolves in the excess of ammonia. More silver nitrate is added cautiously until the precipitate just reappears and the solution after shaking remains slightly opalescent.

If a deeper degree of impregnation is required it can easily be produced by repeating the process from No. 3 (the mordanting) onwards.

Multiplication takes the form of simple binary fission. Usually division is seen in the longer forms, and it may be preceded by the outgrowth of flagella at the point of ultimate separation. The body becomes narrowed and may almost appear to be hinged, as it were, the halves remaining attached but lying at an angle to each other. Somewhat infrequently forms are encountered dividing—in exactly the same manner as above—not into two but into three or more portions.

Cultivation. Cultivation of *S. minus* has been reported by Futaki *et al.* (1917) on Shimamine's medium and by Joekes (1925) on a medium composed of a horse-serum slope covered with Vervoort's medium. The cultural forms as described by Futaki and his co-workers differed from the blood forms of the parasite to a marked degree. McDermott, Schockaert, and Knowles and Das Gupta have all attempted to repeat Joekes experiments but without success. There is need for further work on this subject.

Geographical Distribution and Vectors.

Rat-bite fever is a disease of widespread incidence and cases have been recorded from nearly every part of the world. It is not a disease of any special country nor is the frequency of its occurrence appreciably affected by climate. The main factor determining its incidence is simply the risk of exposure of the human race to the bites of rats. Records have been given by various workers in different parts of the world showing the ratio of infected to apparently non-infected rats. These figures, however, are in the main of little value, for, as will be seen later, it is not so much the rat carrying a heavy infection in its blood-stream (although, no doubt, such a one is also infective) as the animal in the so-called tertiary stage with infected lachrymal, nasal or bronchial secretion which is to be feared. Thus, in Japan, where the houses and the living conditions are such that the rats come more easily into contact with human beings and bites are therefore more frequent, a greater number of cases have been reported than in other countries.

Although the rat is unquestionably the commonest vector of the disease, experiment has shown that the mouse is probably a much more susceptible animal. Natural infections are continually occurring in tame mice kept in the laboratory, and, in their blood the infection becomes very heavy. Mice, however, rarely bite man, and if they do the bite is seldom enough to cause more than a slight abrasion of the skin. Cases have

followed the bites of cats, dogs and ferrets, while scratches from cats and other animals have produced a like result, but such instances are more than likely merely examples of simple mechanical transmission, the infective material being carried on the teeth or claws of the transmitting animal, which may have been in contact immediately before with a dead mouse or rat.

As a rule the saliva of rats is rarely infected and *Spirilla* cannot be discovered microscopically in this secretion. To explain the infectivity of the bite the theory was advanced that small cuts and scratches on the gums of the infected animal permitted the contamination of the saliva by the blood. Mooser (1924) suggested an alternative mechanism. He drew attention to the eye symptoms, the conjunctivitis, keratitis, iritis and palpebral œdema occurring in rats towards the end of the infection and also noted that the lachrymal secretion in such cases contained *Spirilla*. Mooser's work has since been confirmed and amplified by McDermott (1928). The latter has discovered that, in addition to the infected secretion coming from the eye, the rats in the 'tertiary' stage of the disease had gumma-like lesions in the lungs, mediastinal lymph glands and in the bronchi, which were liable to ulcerate and allow the *Spirilla* to escape into the bronchi. On the infrequent occasions when reliable information has been forthcoming with regard to the general appearance of the rat whose bite had caused infection in man the description has tallied fairly closely with that of a rat in the tertiary stage.

Pathology.

The final proof that *Spirillum minus* is the causal organism of rat-bite fever was provided by Theiler and others (1926) and by Schockaert (1928) who produced the disease in man. The latter inoculated *Spirilla* from two sources, one of which was a case of rat-bite fever or sodoku in man and the other a natural animal infection. This also goes to prove that the 'natural' infections in animals are not caused by a distinct species or strain incapable of infecting man as was suggested might be the case by Kasai (1921). The injection of the virus into man was made as a therapeutic measure in cases of general paralysis of the insane. The results of this form of treatment, however, are probably not so good as those produced by malaria.

The course of the disease in these induced cases does not differ from that in cases acquiring their infection by a bite, except that the pain and other discomforts of the primary lesion and the lymphadenitis can be avoided by making the inoculation into a vein, at the same time taking precautions not to allow infected material to come into contact with the skin or other tissues.

There are but few accounts of the pathology of this disease in the literature and there is room for much more work on the subject. Such accounts as are available show that, apart from the primary sore, there is very little which would not be found in any case due to fever. There

appears to be a degree of cachexia and hyperæmia of the viscera. Parenchymatous degeneration of the liver and kidneys may occur (Kaneko and Okuda). Signs of a catarrhal gastro-enteritis from involvement of the gastric and intestinal mucosa may be found, especially in those cases where diarrhoea has been a symptom. The heart may show some slight myocarditis and there may be some hyperæmia of the brain and meninges.

Before leaving this question of the pathology the fact must be noted that in certain cases following the bites of animals infection may be produced by a *Streptothrix*. Schottmüller (1914) found two species of that organism, *Streptothrix muris ratti* and *S. taraxeri-capapi*, in cases following the bite of a rat and a South African squirrel respectively. Blake (1916) isolated a similar *Streptothrix* from the vegetations of an ulcerative endocarditis accompanied by myocarditis and, in addition, degeneration and necrosis of the cardiac muscle. Tunnicliff and Mayer (1918) also recorded a similar case in man, and previously Tunnicliff (1916) had shown that a *Streptothrix* might occur in the broncho-pneumonia of rats. It is thus highly probable that two distinct diseases may be transmitted to man by the bite of animals, the true rat-bite fever or sodoku and the ulcerative endocarditis due to the *Streptothrix*, either of which may serve to mask, or equally may be mistaken for, the other.

The Disease in Animals.

Mice, white rats, guinea-pigs, rabbits, cats, ferrets and monkeys (*Macacus rhesus*) are included in the list of susceptible animals.

Mice. Mice are probably the most susceptible of all the animals to infection with *S. minus*, and, indeed, the most reliable method of diagnosis in human cases in which, for some reason, there may be difficulty in finding the specific organism by microscopical means, is to inject mice with blood obtained from the human patient during one of the bouts of fever. There is one fallacy which has to be guarded against—the frequency with which infections may occur even under laboratory conditions in tame mice. Variations occur in the virulence of the infecting strain and sometimes it may become highly pathogenic. This is of practical importance when these animals are being used for some other purpose, such as experiments with *Spirochæta recurrentis* or *Trypanosoma cruzi*.

The incubation period in mice is from 3 to 15 days according to the virulence of the strain. Once a strain has become established, the incubation period, as well as the entire course of the disease, tends to become more or less constant. The average incubation is about 8 to 10 days, after which the organisms can be detected in increasing numbers in the peripheral blood. They may persist for some weeks and relapses sometimes occur. McDermott states that six and a half months after the original inoculation the infection can be successfully transmitted to a fresh host. The numbers present in the blood vary from day to day and there is also a tendency for certain forms, e.g. either long, medium or short forms, to be in numerical preponderance on any given day

(Robertson, 1924). In mice, according to Schockaert, the pathological changes are less evident than in rats, but otherwise the disease is much the same. The question of the hereditary transmission of the disease in mice has been investigated by Troisier and Clement, and by Salimbeni, Kermorgant and Garcin (1925), all of whom incline to the view that it may occur, but Abe (1924), Worms (1926) and McDermott (1928) tend to the opposite opinion.

Rats. In the rat the course of the disease may be divided into several well-marked stages, in this respect being similar to syphilis in man. McDermott describes the following stages :

(1) Incubation period.

(2) Primary stage. In this the *Spirilla* are found only in the skin wound and the lymphatic system.

(3) Secondary stage. The onset of the secondary stage is synchronous with the appearance of the organisms in the blood and may come on within from 4 to 30 days after the inoculation. The secondary stage lasts from 4 weeks to 4 months, after which it is no longer possible to inoculate the blood of the rat successfully into mice.

(4) Latent stage. The peripheral blood is free from parasites, and so far as can be seen symptoms are in abeyance.

(5) Tertiary stage. After three and a half to four and a half months, conjunctivitis, keratitis, iritis and œdema of the eyelids may be found. The lachrymal secretion contains *Spirilla* (Mooser, 1924). Gummatoid lesions may occur in the lymph glands, especially those in the mediastinum, and occasionally in the liver and spleen. There is first a hyperplasia and proliferation of the reticulo-endothelial cells and this is followed by necrosis. In the lesions of longer standing there is a central zone of necrotic material surrounded by a zone or capsule of fibrous tissue. The necrotic material contains *Spirilla*, for, if inoculated into a mouse, infection will result.

Guinea-pigs. Strains of *S. minus* isolated from human cases vary very much in their virulence towards guinea-pigs. Ogata, Mooser and Ruys, among others, ascribed a considerable degree of pathogenicity to the strains they isolated, whereas neither Row, nor Parmanand nor Robertson was able to find any signs of pathogenicity. McDermott found that after a few passages his strain became stabilized. The incubation period was about 9 days and the death of the animal occurred in about 28 days. Conjunctivitis and keratitis were noted and also hyperplasia of the lymphoid and endothelial elements in the glands and in the spleen. This endothelial and lymphoid proliferation was followed by progressive fibrosis. Areas of alopecia accompanied by œdema of the skin in certain parts—the eyelids, nostrils, lips, bases of the ears and genitalia—were observed by Schockaert.

Rabbits. Intrascrotal injections were carried out in rabbits by Matsumoto and Adachi (1923) and in 3 to 5 days a lesion appeared very similar to that of rabbit syphilis. Mooser (1925) noted an instance in

which transmission took place from an infected doe with œdema of the vulva to a male rabbit during coitus. The primary lesion formed on the prepuce 15 days later and shortly afterwards the general infection developed.

Other animals. The sequence of events in inoculated monkeys is similar to that in man (Futaki and others, Kobayashi and Kodama, Ruys and others). Cats are also susceptible, as was proved by Mooser, Kasai, and by McDermott. This last worker also infected ferrets.

Serology.

The Wassermann reaction is positive in a proportion of human cases but its diagnostic value is doubtful. Knowles and Das Gupta, for example, found one doubtful, two 'moderately' positive and six definitely negative out of their nine cases.

On the other hand, there seems to be direct evidence of the formation of lytic substances in the serum although the different strains show marked variations in their ability to produce lysins. Kusama and Kodama (1919) isolated two divergent strains from rats, one of which, inoculated into monkeys and guinea-pigs was definitely pathogenic and produced substances lytic to both strains, whereas the other (although infection probably took place, for afterwards injections into the same animals with the more virulent strain were negative) did not produce a demonstrable infection nor did the serum possess a lytic activity. Of three strains from man, a wild mouse and a tame mouse, Kasai found that those infecting man and the wild mouse produced lytic substances active against all three strains, but that the one from the tame mouse caused the formation of lysins active only against the same strain. Schockaert states that the immune bodies are almost exclusively lysins which are very active when undiluted. Dilution of 1 in 10 to 1 in 20 makes the action very feeble, and of 1 in 100 renders the effect unappreciable. As a measure of control Schockaert carried out inoculations with blood containing *Spirilla* which had been subjected to the action of the lytic sera for one hour. The undue prolongation of the incubation period or the failure to produce infection was indicative of the active nature of the lysins.

In man Schockaert found that the lysins appeared in the serum between 14 and 30 days after the first attack of fever. Schockaert was working with two strains, the first from a sodoku case and the other one of the natural infections. The former produced sera almost equally active against both strains, whereas the latter (i.e. the mouse strain) exhibited a degree of potency only against the specific organisms and was very feeble in its lytic action against the human variety. Infection with either the human or the mouse strain protected against a second infection of the same or the other organism.

Guinea-pigs, at a late stage in the disease, have been shown by McDermott to produce antibodies which, in dilutions up to 1 in 8, were capable of immobilizing the *Spirilla* in the blood of mice in one hour.

Apparently it did not matter whether the strain was human or animal in origin. Rabbits also produce lysins in their serum.

To sum up, there is evidence that one attack of the disease will protect against subsequent attacks, that this is presumptive, to a certain extent, of the specific identity of the various strains, and that during convalescence antibodies in the nature of lysins are formed in the serum, active when only slightly diluted but rapidly losing their potency in dilution.

Treatment.

Since the discovery in 1912 by Hata that salvarsan acted as a specific cure, work confirmed by Surveyor (1913) and Dalal (1914), many other drugs have been tried but nothing has so far been found which holds out any advantages over salvarsan and its derivatives. One injection in human cases of 0.4 to 0.6 gm. of novarsenobillon is usually enough to effect a complete cure. Bismuth and mercury preparations have been tried but do not appear to have much action. Small doses of arsenical preparations do not cause the production of arsenic-resistant (drug-fast) strains. Some work has been done on the curative effect of immune sera but as yet too little is known of this.

REFERENCES.

Only the more important references are included ; for more complete lists the monographs of McDermott (1928), Schockaert (1928) and Robertson (1924) should be consulted.

- ADACHI, K., 1921, *J. Exp. Med.*, **33**, 647.
 BLAKE, F. G., 1916, *J. Exp. Med.*, **23**, 39.
 BORREL, A., 1905, *C.R. Soc. Biol.*, Paris, **58**, 770.
 BREINL, A. & KINGHORN, A., 1906, *Mem. Lpool. Sch. Trop. Med.*, **21**, 55.
 CARTER, H. V., 1887, *Sci. Mem. Med. Off. Army Ind*, Part **3**, 45.
 FUTAKI, K., TAKAKI, I., TANIGUCHI, T. & OSUMI, S., 1917, *J. Exp. Med.*, **25**, 33.
 HATA, S., 1912, *Münch. med. Wschr.*, **59**, 854.
 ISHIWARA, K., OHTAWARA, T. & TAMURA, K., 1916, *Tokyo Igakkwai Zasshi*, **30**, 52.
 JOEKES, TH., 1925, *Lancet*, Lond., ii, 1225.
 KASAI, K., 1921, *Chuwo Juikwai Zasshi*, **34**, No. 7.
 KNOWLES, R. & DAS GUPTA, B. M., 1928, *Ind. Med. Gaz.*, **63**, 493.
 McDERMOTT, E. N., 1928, *Quart. J. Med.*, **21**, 433.
 MOOSER, E., 1925, *J. Exp. Med.*, **42**, 539.
 PARMANAND, M. J., 1923, *Ind. J. Med. Res.*, **12**, 609.
 ROBERTSON, A., 1924, *Ann. Trop. Med. Parasit.*, **18**, 157.
 ROW, R., 1917, *Ind. J. Med. Res.*, **5**, 386.
 SCHOCKAERT, J., 1928, *Arch. int. Méd. exp.*, **4**, 133.
 THEILER, M., 1926, *Amer. J. Trop. Med.*, **6**, 131.
 TUNNICLIFF, R., 1916, *J. Infect. Dis.*, **19**, 767.
 WENYON, C. M., 1906, *J. Hyg.*, Camb., **6**, 580.

CHAPTER IX. THE LEPTOSPIROSES.

BY EDWARD HINDLE,

THE BEIT RESEARCH FELLOW IN TROPICAL MEDICINE (WELLCOME
BUREAU OF SCIENTIFIC RESEARCH, LONDON).

General Account.

THE spirochætes grouped together under the subgenus *Leptospira* have the form of a slender, cylindrical and highly flexible filament, with very tightly wound, regular, and rather shallow spirals. Each extremity gradually tapers to a point and in life is often bent over in the form of a hook. In some forms the spirals are so close together that the distance between them is impossible to measure, but especially in the saprophytic forms the spirals are more distinct. When the organism is dying the spirals often become unwound and then give the appearance of an ordinary spirochæte.

The terminal portion of the body seems to be much more motile than the middle and the lashing about of the hooked or curved extremities, combined with the very tight spiral, is one of the most characteristic features of these organisms when observed living by means of dark-ground illumination (Figs. 19, 26 and 27, facing pp. 139, 140). When these crooked ends are rapidly rotated the optical appearance of a swollen extremity may be produced, but later the organism may straighten out into a flexible spiral filament. Without dark-ground illumination it is almost impossible to distinguish the spirals, as each turn is only about half a micron in length, and possibly as a result of this difficulty in seeing their structure, the first of these organisms was not described until 1914, when Wolbach and Binger (1914) gave an account of *Spirochæta biflexa*, an organism found in fresh water. Their importance, however, was not fully realized until Inada and Ido (1915) described a similar form under the name of *Spirochæta ictero-hæmorrhagiæ* as the causal agent of Weil's disease in Japan. Since that date these organisms have been found in a great variety of sites, living either saprophytically or parasitically, and *Leptospira* is probably one of the most widespread subdivisions of the spirochætes and is of considerable importance from a pathogenic point of view.

The average length of these organisms varies from 7 to 14 μ , but chains of individuals may be formed resulting in the production of very long forms. The diameter is difficult to estimate, but is generally given as about 0.25 μ . Each spiral is about 0.5 μ in length and 0.3 μ in depth, consequently there are usually about twice as many spirals as the number of microns in the total length. The spirals are very uniform and in life do not change their shape.

✓In stained preparations the whole body takes a uniform coloration, there being no evidence of the existence of an axial filament or cell membrane, and also the presence of volutin is doubtful.

✓One of the most distinctive features of *Leptospira* is its susceptibility to various digestive ferments and resistance to saponin and distilled water. Whereas ordinary blood spirochaetes resist tryptic digestion for many days, *Leptospira* is completely dissolved in a few hours. Similar results are obtained by the action of 10 per cent. bile-salts, which easily dissolve *Leptospira* but have a much slower action on other spirochaetes. Ten per cent. saponin, on the other hand, immobilizes ordinary spirochaetes within half an hour, laying bare the axial filament in some species, but *Leptospira* is completely resistant. Similarly, distilled water, which rapidly produces plasmolysis of all other forms, including *Cristispira*, has no effect on *Leptospira*.

As in the case of the blood spirochaetes there are no satisfactory morphological differences between any of the members of this group, and recent work tends to support the view that at any rate all the pathogenic forms may be merely varieties of the almost ubiquitous water *Leptospira*. The writer is of the opinion that this view is essentially correct, consequently any study of these organisms should begin with an examination of the common water form.

***Spirochaeta (Leptospira) biflexa* Wolbach & Binger, 1914.**

[Synonyms : *Spirochaeta pseudo-icterogenes (aquatilis)* Uhlenhuth and Zuelzer, 1921. *Spirochaeta pseudo-icterohæmorrhagiæ* R. Vincent, 1926.]

This spirochaete seems to be almost universally present in fresh and marine water although it can only rarely be detected without using some culture method, except in water containing much decaying organic matter. It was first described, under the name *Spirochaeta biflexa*, by Wolbach and Binger (1914), who found it developing in the filtrate of water from a pond in Boston, U.S.A., that had been passed through a Berkefeld V filter. Later these spirochaetes, under various names, have been recorded from most parts of the world and have been studied in particular by Zuelzer, either alone or in collaboration with others.

These water forms undoubtedly live saprophytically, and if the amount of suitable organic matter in the water can be increased, whilst the pH does not fall below about 7·0, a rich growth is generally obtained. Various methods have been described, but the simplest is the writer's faecal culture, by means of which the organisms were first demonstrated in London tap-water (Hindle, 1925). A portion of human faeces, about the size of a pea, is mixed with about 20 c.cm. of tap-water in a Petri dish and kept at a temperature of 25 to 30° C. Usually an abundant growth of *Leptospira* will be found after ten days, although the maximum growth is not reached until the third or fourth week, after which the numbers gradually diminish. A pure culture can be obtained by passing through an L3 Pasteur-Chamberland filter, which arrests the ordinary bacteria but allows the

passage of the spirochaetes. When freed from other organisms the water *Leptospira* can be cultured in a variety of ways (see below): one of the simplest methods is by the use of serum water (Fletcher, 1928), which consists merely of 5 to 7 c.cm. of sterilized water containing 0.5 c.cm. of melted 2.5 per cent. nutrient agar and 1 c.cm. or less of rabbit's serum. Good results, however, can be obtained with very dilute alkaline agar, or an alkaline solution containing less than 5 per cent. of gelatin. A dilute alkaline broth containing 0.1 per cent. potassium nitrate, but without sodium chloride, is also an excellent medium.

It is possible to find *Leptospira* in water without having resort to cultural methods, but only in the presence of growths of organic matter, such as the slime at the bottom of stagnant pools, or the mucoid material which often collects around the opening of a dripping tap. It has also been observed in slime on the roof of coal mines (Buchanan, 1924). The presence of H_2S in the water is often correlated with a plentiful growth of spirochaetes, and in nature any stagnant waters which smell of sulphuretted hydrogen often contain numerous *Leptospira*. It should be noted that the strains of *Leptospira* occurring in water belong to various distinct serological types. In Kiew, for example, Basilewsky (1930) found that out of four strains of *Leptospira* isolated from local water supplies, three belonged to serologically distinct types, and these were distinct from two other strains isolated by Schüffner and Angerer, respectively. In addition to these serological differences, Damon and Hampil (1929) found that *Leptospira* isolated mainly from Baltimore or Washington waters could be divided into two groups, one capable of growing in cultures kept at 37° C., whilst others would only grow at room temperature. Recently, I have found *Leptospira* in the thermal springs at Dax, which grow at 50 to 60° C. and disappear when kept at 30° C.

THE PATHOGENICITY OF WATER LEPTOSPIRA.

Direct evidence. There have been numerous observations indicating that the inoculation of these organisms (generally after prolonged cultivation) into guinea-pigs or other animals is sometimes followed by the development of a peculiar type of hæmorrhagic jaundice (Weil's disease) accompanied by the presence of *Leptospira* in the blood and internal organs of the host. The most striking example is that described by Buchanan (1924) who found that the slime on the roof of a mine in East Lothian contained *Leptospira*, and when 4 c.cm. of this slime was inoculated intraperitoneally into two guinea-pigs they both died of typical Weil's disease. As an outbreak of this disease had occurred amongst the miners of the locality there can be little doubt that the infection had been derived from the mines.

Zuelzer (1922) was the first to show that the ordinary water *Leptospira* might become pathogenic. Six strains were isolated from water, and of these one was pathogenic and produced Weil's disease when inoculated into guinea-pigs. The strain was also pathogenic to mice and maintained

its properties when subcultured in artificial media. Other observations have been made confirming the view that under certain conditions water *Leptospira* may become pathogenic, and Baermann and Zuelzer (1928), working in Sumatra, were able to bring convincing evidence in support of the water origin of Weil's disease and probably also of other leptospiral infections in man and animals. At that time nine strains of free-living water *Leptospira* had been made pathogenic to animals in various ways, including a water strain obtained from Schüffner known as W A Z, which had been kept in the laboratory for a considerable period. Two c.cm. of a fresh culture of this strain were inoculated (subcutaneously, intracutaneously and intravenously) into a woman. After four days this woman showed a slight rise in temperature for six days without any other clinical signs. Blood was collected from the patient every day by vein puncture and inoculated into guinea-pigs. One of these animals inoculated with blood collected on the sixth, seventh and eighth days showed typical symptoms of jaundice after an incubation period of 26 days. The animal was killed but no *Leptospira* found, although typical lesions of Weil's disease were present. The blood and organs were inoculated into three other guinea-pigs which all showed *Leptospira* on the third day and all died on the sixth day with typical symptoms of Weil's disease. In this experiment, therefore, a typical water strain was shown to be capable of producing infection in a human subject. It is interesting to note that the patient's serum agglutinated and dissolved the original strain but had no effect on the strain after it had been passed through guinea-pigs, and also had no effect on any other strain that was tested.

Okell and Houston (1926) made a very extensive series of tests on the pathogenicity of strains of water *Leptospira* isolated from the various London water supplies. Hindle's coprozoic method of cultivation was used and the resulting organisms inoculated subcutaneously and percutaneously into guinea-pigs and rats. Only 1 out of 23 strains from different sources produced infection, and this in only 1 out of 43 cultures, a good example of the difficulties of investigating a problem of this nature.

Bessemans and Thiry (1928, 1930) injected cultures of Ghent tap-water in Vervoort's medium into guinea-pigs but with negative results. With the fourteenth subculture attempts were made to infect mice, and 4 out of 7 inoculated subcutaneously died after 14 to 27 days, and 1 out of 4 inoculated intraperitoneally. Subinoculations into other mice were successful, but not into guinea-pigs. The infected mice showed *Leptospira* in the blood and urine and at autopsy the organisms could be recovered in pure culture from the bladder. They were common in the kidney but absent from the other organs.

Indirect evidence. In addition to these examples, which afford direct evidence showing that strains of water *Leptospira* in nature may be pathogenic, or acquire pathogenic properties, there is a considerable amount of indirect evidence pointing to the same conclusion.

Cases of Weil's disease generally occur in persons who have been exposed to the chance of contamination from water favouring the growth of *Leptospira*. Tohyama (1927) found that in Japan *Leptospira* thrives in the water of paddy fields, and that coolies working in these fields often suffer from Weil's disease. When calcium cyanamide, which readily kills the spirochætes, was used as a fertilizer, none of the coolies became infected, whilst in the surrounding fields where ordinary fertilizers were used, large numbers of workers acquired Weil's disease. Similar evidence has been brought forward by Sardjito and Zuelzer (1929) in an attempt to correlate the prevalence of Weil's disease with the number of *Leptospira* in the water. In Europe these organisms are much more restricted in their prevalence and growth than in Sumatra, and Weil's disease is much less common. On the East Coast of Sumatra *Leptospira* are found in every kind of water, and cases of Weil's disease occur the whole year round. In the neighbouring island of Java the waters on the West Coast were found to be almost free from *Leptospira* and Weil's disease is very rare. This presence or absence of *Leptospira* was correlated with differences in the pH of the water. The optimum seems to be about 7·3 to 8·0 at a temperature of 28 to 30° C., conditions realized in Sumatra, but not in Java, where the pH of most of the waters was about 6·0.

Many cases of Weil's disease occurred during the War amongst the troops who were frequently exposed to the chance of contamination from the stagnant water in and around the trenches, which must have contained numerous spirochætes. The possibility of the water being contaminated with the urine of infected rats is also a likely contingency in this case, and also whenever these animals have free access to water supplies.

The only properly diagnosed indigenous case in London is that of a seaman who fell into the Thames near Tilbury, and, being half-drowned, swallowed a large quantity of the foul Thames water (Manson-Bahr, 1922). A week later he developed a typical attack of Weil's disease, and *Leptospira* were isolated from his blood by Wenyon and Brown. A subsequent examination of the Thames water near Tilbury showed that *Leptospira* were present in large numbers (Hindle, 1925).

Various epidemics in Europe have been traced to insanitary bathing establishments, amongst which may be mentioned an outbreak near Magdeburg described by Körner (1925) which was arrested by closing certain bathing sheds. The Vesle outbreaks in France were traced to the river and *Leptospira* were found by Etchegoin (1924) in the river water and mud. The disease has also been recorded from among workers in sewers, or in persons who had fallen into filthy water. Kathe (1928) also brings evidence to support the view that slime fever (Schlammfieber) in Germany is an abortive form of Weil's disease and that the infection is generally acquired during bathing or swimming.

Although the evidence in support of the water origin of these infections is so strong, the fact that wild rats are very often infected with this disease and pass out the organisms in their urine has lent support to the view

that these animals are responsible for its transmission to human beings. On the existing evidence, however, it is difficult to assess the relative importance of these two factors; although there are clear indications that conditions favouring the growth of water *Leptospira* are correlated with an increase in the number of cases of infectious jaundice quite apart from the chance of contamination by infected rats.

Infectious Jaundice and other Leptospiral Infections.

Leptospira have been recorded as the causative agent of a number of human and animal infections, and usually one or more specific names have been given to each strain associated with the production of certain clinical symptoms. It is very difficult to distinguish these strains except by immunological tests, but in the following pages the names applied to them have been retained for convenience of description.

Spirochæta (Leptospira) icterohæmorrhagiæ Inada and Ido, 1915

(Figs. 19, 26 and 27, facing pp. 139, 140).

(Synonyms: *Leptospira icterohæmorrhagiæ*, *Spirochæta icterogenes*, *Leptospira icterogenes*, *Spirochæta nodosa*, *Leptospira icteroides*.)

This organism was first isolated from cases of Weil's disease in Japan by Inada and Ido, who described it in January, 1915, under the title of *Spirochæta icterohæmorrhagiæ*. It was independently discovered in Germany by Uhlenhuth and Fromme (1915), who gave it the name of *Spirochæta icterogenes*, and by Huebener and Reiter (1915) who used the term *Spirochæta nodosa*. The parasite is still commonly referred to in German publications as *Spirochæta icterogenes*, but the name suggested by Inada and Ido has priority. The morphology of the spirochæte is identical with that of the water *Leptospira* described above (p. 297), from which it can only be distinguished by its serological properties.

CULTIVATION.

Media.

The parasite can readily be cultivated on a variety of media, of which the following are among the more convenient:

Fletcher's medium. Five to 7 c.cm. of sterile tap-water or distilled water is mixed with 0.5 c.cm. of a melted 2.5 per cent. nutrient agar and 1 c.cm. or a little less of rabbit's serum in a test-tube, and the pH brought to 7.7. When making primary cultures, about 0.5 c.cm. of the patient's (or animal's) blood is inoculated into each tube and the cultures kept at a temperature of about 28° C. (Fletcher, 1928). The organisms can rarely be detected in the cultures before the fifth or sixth day, and it is advisable to leave them at least a week to obtain a plentiful growth. Subcultures should be made as soon as the numbers of spirochætes begin to decrease.

Noguchi's serum medium. This medium was described by Noguchi and Lindenberg (1925) and has the following formula :

Saline solution (0·9 per cent.)		800 parts.
Fresh rabbit-serum		100 „
Nutrient agar (2·0 per cent., pH = 7·2) ..		100 „
Rabbit hæmoglobin solution (made by taking 1 part of defibrinated rabbit's blood in 3 parts of distilled water)		10 to 20 „

Growth occurs at the top of this medium as a whitish scum composed of a mass of the organisms. This method of cultivation is one of the best for obtaining large numbers of spirochætes.

Noguchi-Wenyon medium (Wenyon, 1926). This medium gives very satisfactory results for ordinary routine purposes. It is most easily prepared as follows :

Normal saline solution (0·85 per cent.)		270 c.cm.
Nutrient agar (2·5 per cent.) pH 7·5 ..		30 „
(= about 6 test-tubes).		

Steam the saline and the nutrient agar slopes until the latter are melted and mix while warm. Then run into test-tubes, 9 c.cm. in each, after which plug and autoclave the tubes. Then cool to about 50° C. and add 15 drops of whole rabbit's blood to each tube. It is important that the blood should *not* be mixed with the agar but merely allowed to run in. Then whilst still warm incubate over-night at 37° C. and finally cap to prevent evaporation. This medium will usually keep from one to two months.

Vervoort's medium (Vervoort, 1923¹). Another medium which gives excellent results with all strains of *Leptospira*. It consists of a solution of 0·1 per cent. peptone and 0·05 per cent. NaCl in tap-water, buffered by the addition of 5 to 10 per cent. of a phosphate solution and adjusted so that the final pH is 7·2. The further addition of 10 per cent. of inactivated rabbit-serum, or one drop per c.cm. of whole rabbit-blood, is said to be advantageous though not essential. The organisms are best cultivated aerobically in small tubes (2 c.cm.) without a layer of paraffin, at a temperature of 28° C.

Isolation of Leptospira in Cultures.

Cases of Weil's disease generally contain *Leptospira* in the circulation during the febrile period, and cultures can be made by taking about 0·5 c.cm. of the patient's blood, drawn from an arm vein, and inoculating it into a tube containing one of the media recommended above. The tubes are kept at a temperature of 28 to 30° C., and should be examined by means of dark-ground illumination after about a week. As a general rule the organisms are not easily seen before the sixth or seventh day, and in some instances two to three weeks may elapse before they are present in sufficient numbers to be found without a very lengthy search. Subcultures are made by transferring one or two loopfuls of the original culture into a

fresh tube. If the patient has already passed through the febrile stage of the disease the organisms can sometimes be recovered from the urine, and in such cases it is preferable to make repeated inoculations into guinea-pigs of centrifuged urine collected on successive days. The primary isolation of cultures from infected guinea-pigs may be effected by inoculating tubes of the above media either with 0·5 c.cm. of heart-blood, or preferably with small fragments of the suprarenal or liver of the animal. When culture tubes are contaminated with other organisms *Leptospira* can often be recovered by filtration through a Berkefeld filter. As a general rule, however, the infection of guinea-pigs affords the simplest method of recovering *Leptospira* free from other organisms, and in addition such animal passages help to maintain the virulence of the cultures, which tend gradually to diminish.

When cultures of *Leptospira* are maintained in the laboratory it is advisable to make subcultures every month, and if it is desired to maintain the virulence of the organisms animal passages should be made every few months.

PATHOLOGICAL EFFECTS.

In Man.

Infection with *S. icterohæmorrhagiæ* produces a great variety of clinical symptoms, ranging from slight jaundice of short duration up to fatal hæmorrhagic jaundice. Different races of the organism possess very varying degrees of virulence, to such an extent that it is almost impossible to find any characteristic clinical features of the disease except with highly virulent strains, such as those observed in France during the War. Under such conditions there is a sudden onset, with rigors, vomiting, headache, diarrhœa and abdominal pains. A few hours later fever ensues with intense injection of the eyes, great prostration and agonizing muscular pains and aching of the bones. The fever is of irregular type between 103 and 105° F., falling by lysis about the tenth or eleventh day. Spirochætes can sometimes be seen in the blood during the early stages of the disease, but the most certain method of diagnosis is to make a culture of the blood collected during the first three days of fever, or to inoculate a guinea-pig with about 6 c.cm. of the blood. In the second or third week of the disease there is usually a secondary rise in temperature associated with the excretion of spirochætes from the kidney. During this period the organisms appear in the urine, and they can sometimes be found as long as 100 days after the original infection.

Convalescence is generally established in the third week. Jaundice occurs in about half the cases and is usually noted two to three days after the onset. It may be accompanied by hæmorrhages into the conjunctivæ or skin, or even from the mucous membranes.

The urine is highly coloured and generally contains albumin and bile, and sometimes casts and red blood-cells. From the fifth to the eighth days of fever it is said to give an intense green reaction when one or two

drops of acetic acid are added. The blood shows a polymorphonuclear leucocytosis, whilst later there is an increase in the lymphocytes.

Martin and Pettit (1919) give the following classification of the various clinical types of this disease :

1. *Cases with jaundice.* (a) Cases with a syndrome of severe jaundice ; (b) Cases with a syndrome of infective jaundice, with febrile relapses, (i) Benign catarrhal cases, (ii) Prolonged febrile cases, (iii) Meningeal cases, (iv) Cases with a nervous syndrome, (v) Pulmonary cases.

2. *Cases not showing jaundice.* (a) Meningeal cases ; (b) Cases with a muscle syndrome.

3. *Spirochaetosis and jaundice associated with typhoid, syphilis or diarrhoea.*

4. *Spirochaetosis without jaundice associated with leucæmia.*

Considerable difficulty is met with in the diagnosis of such obscure infections as slime fever, mild epidemics of jaundice, &c. Certain authors, e.g. Kathe (1928), consider that the so-called slime fever, which sometimes occurs in epidemic form in Germany and other European countries, is merely an abortive form of Weil's disease, and that infection is generally acquired during bathing or swimming. Although spirochætes have sometimes been found, the evidence for regarding all these mild cases of febrile jaundice as spirochætal in origin is very unsatisfactory. In Great Britain a few isolated cases of spirochætal jaundice have undoubtedly occurred, such as those described by Manson-Bahr, Wenyon and Brown (see Manson-Bahr, 1922) and Lyon (1925).

In addition, extensive epidemics of infectious jaundice have occurred from time to time in various parts of the country which in their general clinical characteristics closely resemble mild cases of spirochætal jaundice. Hitherto, however, no satisfactory evidence has been obtained in support of their spirochætal nature, and numerous bacteriological and serological investigations have given uniformly negative results. The spirochætal nature of similar epidemics on the continent, therefore, must still be regarded as somewhat uncertain, and it is not improbable that there are at least two distinct types of infectious jaundice, in one of which the ætiological agent is not *S. icterohæmorrhagiæ*.

Pathological Effects in Animals.

Guinea-pigs are particularly susceptible to infection with *S. icterohæmorrhagiæ* and may readily be infected by rubbing a little infective material on the surface of the skin, or conjunctiva. This percutaneous method is even preferable to subcutaneous or intraperitoneal inoculations, except when using material containing very few spirochætes, such as when testing the blood or urine from human cases.

The animal generally shows a rise in temperature towards the end of the first day after inoculation, and fever persists during the disease until shortly before death. Infected guinea-pigs show a very intense jaundice which only develops towards the end of the disease when the temperature

falls. The colour is such that the animal often looks as if it had been soaked in picric acid. In addition to jaundice the urine contains albumin and casts, and often red blood corpuscles. Spirochætes can generally be found in both the blood and urine, especially towards the end of the disease. Hæmorrhages are generally observed after the first three or four days and may occur in the nasal regions or in the neighbourhood of the anus or female genitalia. They are never abundant, but merely redden the surface.

The infection is almost invariably fatal in guinea-pigs, but the duration of the disease varies according to the virulence of the strain. As a general rule animals inoculated with *passage* virus die in 4 to 8 days, whilst those inoculated from human cases may live for 10 days or longer.

Most other laboratory animals are refractory to ordinary doses of the spirochætes; among those which have been tested may be mentioned the lower monkeys, the cat, pig, horse, sheep, hedgehog, rabbit, white mouse, white rat, fowl, pigeon and frog. In the case of *Macacus* monkeys, rabbits and tame rats and mice, the inoculation of large doses may produce infection, but death is always very exceptional.

On the other hand, in certain animals, particularly wild rodents, the spirochætes may persist for considerable periods without producing any ill effects. Such animals may serve as reservoirs of the virus, for commonly the spirochætes invade the kidneys and during long periods are passed out in the urine. Among the rodents which have been found infected in nature may be mentioned *Mus decumanus*, *M. alexandrinus*, *M. rattus*, *Microtus montebelloi*, field mice (*Apodemus*), and a fox. Wild rats, however, are by far the most important reservoirs, and have been found to be infected in varying proportions in most parts of the world where they have been examined. In London, Stevenson (1922) found that about 30 per cent. of the wild rats harboured *S. icterohæmorrhagiæ* in their kidneys. Foulerton (1919), also in London, found only 4 infected out of 98 brown rats, whilst Coles (1918), in Bournemouth, found 9 infected out of 100. Buchanan (1927), during the East Lothian epidemic, found 61 out of 166 infected, and Middleton (1929), in Oxford, 41·7 per cent. out of 235 examined. In the latter case very young rats were found to be free from infection, and the incidence of infection increased up to a maximum in fully grown individuals, after which there was a slight diminution in the oldest rats. Similar results have been obtained in most parts of the world where rats have been examined, but the percentages of rats found to be infected have shown considerable variations. It is evident, however, that these animals act as an efficient reservoir for the spirochæte in a form virulent to guinea-pigs. It seems likely, therefore, that they may help to spread the disease by contamination of water or foodstuffs with urine containing the spirochætes.

The common domestic mouse, on the other hand, although easily infected by the inoculation of virulent material, and showing the same persistence of spirochætes in the kidneys and urine, does not seem to acquire the infection in nature. This marked difference between rats and

mice in the incidence of this disease strongly supports the view that the infection is primarily acquired from water, as rats are well known to frequent sewers and drains containing foul water, whilst house mice do not seem to require water and do not frequent its neighbourhood. It is a curious fact that although both wild rats and mice are very susceptible to this infection, white rats and white mice are generally refractory. Pettit failed to produce any signs of infection by the inoculation of large doses of a virulent strain of the disease. Inada, Ido, Hoki, Kaneko and Ito (1916) record having produced jaundice in 4 out of 14 white mice inoculated with a Japanese strain of *S. icterohæmorrhagiæ*, but in the only one examined no spirochætes could be found in the liver. Okell and Houston (see above, p. 299), however, succeeded in infecting rats with a strain of water *Leptospira*.

Tame rabbits are also very difficult to infect, but Oba (1923), in Japan, has found that by using rabbits weighing less than 1,000 gm. and inoculating the spirochætes into the liver, every animal becomes infected. The inoculum is prepared by making an emulsion of the liver of an infected guinea-pig and centrifuging to remove cell particles, so as to leave a suspension of spirochætes as free as possible from other material. As a general rule infected rabbits show signs of jaundice after 3 to 5 days and the symptoms persist for 2 to 7 days, during which the animal does not eat. A rise in temperature accompanies the appearance of jaundice. About half the animals recover from the infection after a long convalescence.

Dogs are well known to be susceptible to this disease, as first shown by Courmont and Durand (1917). They found that puppies could readily be infected by the inoculation or ingestion of the spirochætes and contracted typical and fatal jaundice. In older dogs the disease was found to be much less typical, which may explain the negative results of Nicolle and Lebailly (1918) and Uhlenhuth and Fromme (see Fromme, 1919), who were unable to infect dogs by intraperitoneal inoculations of infective material. Monti (1916) and Sani (1919) also found that dogs were susceptible, but the most extensive series of experiments with these animals are those recorded by Okell, Dalling and Pugh (1925). They investigated the question of enzootic jaundice in dogs in Great Britain, and as a result of the study of various outbreaks, especially amongst sporting dogs, were able to show that this disease is caused by a spirochæte indistinguishable from *S. icterohæmorrhagiæ*. In addition to producing the infection in puppies by the inoculation of material from naturally occurring cases of the disease in dogs, they also inoculated puppies with a strain obtained from infected rats in Scotland. The post-mortem appearances of dogs infected with this strain were identical with those dying from the natural disease, or as a result of the inoculation with a strain of canine origin. The fox in England has also been found infected in nature (Dunkin and Laidlaw, 1926).

Krumbein and Frieling (1916) reported a case of two officers living in a room with a dog suffering from jaundice, who both developed what

seemed clinically to be typical spirochætal jaundice, therefore it is probable that the dog may occasionally act as a carrier of the infection. In addition to the foregoing much has been written on the subject of 'Stuttgart dog disease', the relation of which to Weil's disease has not been definitely proved. Lukês (1924) and Křiváčěk (1924) found spirochætes morphologically identical with *S. icterohæmorrhagiæ* in the tissues of dogs that had died from this disease. This spirochæte, named by Klarenbeek (1928) *Spirochæta ictero-uræmia canis*, occurs especially in the kidneys and is passed out in the urine. The organism is pathogenic to guinea-pigs, in which it reproduces the symptoms of Weil's disease, though of a milder type than that produced by human strains. Serological tests show that there is a very close relationship between the dog spirochæte and both human and rat strains of *S. icterohæmorrhagiæ*, and in view of this general similarity it seems most probable that Stuttgart disease is merely Weil's disease in dogs.

Monkeys. Comparatively few experiments have been made with monkeys. Hübener and Reiter (1915) record that their inoculation with this virus simply produced a short rise in temperature and no other effects. Noc (1920), however, observed the death of a *Cercopithecus patas*. Martin and Pettit (1919), in their earlier work, failed to infect monkeys, but later Stéfanopoulo (see Pettit, 1928), by the inoculation of the enormous dose of three-quarters of the liver of an infected guinea-pig, succeeded in producing jaundice in a *Macacus sinicus*, which, however, recovered from the attack. Spirochætes were not found in this animal, but its blood contained agglutinins and protective antibodies against the infection.

Wilbert and Delorme (1927) described an outbreak of this infection amongst chimpanzees at Kindia, French Guinea. They named the causative organism *Spirochæta anthropopithecii*, but their results, including serological tests, clearly indicate that it was identical with ordinary strains of *S. icterohæmorrhagiæ*.

General Pathological Changes Produced.

Although this disease is almost invariably fatal in guinea-pigs, in man the mortality varies from approximately 5 per cent. in Europe to 33 or even 57 per cent. in Japan. The pathological changes in the two are very similar.

At autopsy the majority of the tissues are jaundiced; œdemata and hæmorrhages are usually present, especially in the fat of the crural and axillary regions, and in the lungs, intestines, adrenals, kidneys and striated muscles. In addition, various histological changes may be observed. The liver shows fatty degeneration, necrosis and slight lymphoid infiltration; and the kidney intense epithelial nephritis with numerous cylindrical casts; the lungs, hæmorrhagic infarcts; the adrenals, hæmorrhages; the spleen, pycnosis and hæmatophagy; the lymphatic ganglia, intense hæmorrhages and hæmatophagy; and the blood, anæmia and myelocytosis.

The mechanism that produces these lesions is unknown, for hitherto it has been impossible to demonstrate the presence of toxins. Although infection with the organism produces first a rise in temperature followed by a fall, the inoculation of large doses (10 c.cm.) of the filtrate of rich cultures produces no effect in guinea-pigs (Pettit, 1928). On the other hand, the inoculation of cultures of low virulence may produce a cachectic state and sometimes death, without the development of any characteristic lesions and also without any spirochætes being visible in the organs.

IMMUNOLOGICAL REACTIONS.

Complement fixation. Costa and Troisier (1916) tested the Bordet-Gengou reaction with the serum of patients infected with spirochætal jaundice and obtained a positive reaction with a syphilitic antigen. Conversely a spirochætal jaundice antigen gave a positive reaction with syphilitic serum. It is evident, therefore, that this reaction is not very specific, but these results might be regarded as indicating a group relationship between the two infections.

Antibodies. The antibodies present in the serum of recovered patients are very specific and persist in the blood for many years. When the spirochætes are mixed with immune serum the majority of them are rapidly destroyed, although isolated individuals may be resistant (see below, p. 309).

Agglutinins and lysins. The presence of agglutinins may be demonstrated in the blood of convalescents at least eight years after recovery from the disease. Normal human serum, and also that of syphilitic patients, has no agglutinating action on this organism, therefore it can be used as an easy method of testing the blood of any person suspected of being infected with spirochætal jaundice. It should be noted, however, that agglutinins do not develop in the serum before the ninth or tenth day of the disease, after the disappearance of jaundice, and in rabbits the maximum concentration is only attained one month after infection. Although the sera of most laboratory animals have no action on *S. ictero-hæmorrhagiæ*, for some unexplained cause the blood of some of the lower monkeys agglutinates this organism. Horses and guinea-pigs also develop agglutinins. In common with most bacterial agglutinins, these are thermo-stable, and serum that had been kept at 70° C. for an hour was found to have lost only 25 per cent. of its agglutinating properties (Oba, 1923).

For the application of this test it is advisable to use young cultures of the same age and to mix them together in order to obtain a uniform suspension. Various dilutions from 1 : 10 up to 1 : 50,000 of the sera to be tested are then prepared. Schüffner and Mochtar (1927), who have made a particular study of this test, found that white porcelain plates made with a series of hollows, such as are used for mixing water colours, were very suitable for making the mixtures of sera and *Leptospira*. Equal quantities of the culture suspension and the various serum dilutions are mixed together on the dish, which is then covered with a bell jar to

prevent evaporation and allowed to stand at room temperature for 16 to 20 hours. The mixtures are then examined by means of dark-ground illumination, a loopful being taken from the various dilutions in their regular order and spread on a slide, care being taken that these loopfuls are as much as possible of the same size. By using low magnifications, e.g. $\frac{3}{8}$ -in. objective and No. 8 eyepiece, the parasites can be distinguished and it is unnecessary to use coverslips. Also 12 to 16 dilutions can be placed on one slide, which saves time and economizes material. With homologous sera the agglutination of the *Leptospira* into clumps or nets only starts with 1 : 10 dilutions, but in 1 : 25 or 1 : 100 is very evident. These clumps swim about, but there are always individual specimens that remain distinct from the agglutinated masses. In the higher dilutions, 1 : 500 to 1 : 10,000 agglutination is superseded by lysis, and the organisms are disintegrated (e.g. after 16 to 20 hours with a dilution of 1 : 5,000). This lytic process is quite different from the reaction in ordinary bacteria, for it is quite independent of complement, and, according to Schüffner and Mochtar, only develops in the higher dilutions. In lower dilutions or in undiluted serum the *Leptospira* remain alive and unaltered. On the other hand, Bonne (see Pettit, 1928, p. 66) obtained both agglutination and lysis with the same dilutions from 1 : 100 to 1 : 10,000. In the same way that some few individuals are not agglutinated, some are not disintegrated by lysis, and retain their usual motility. It might have been anticipated that cultures made from these individual survivors would also be more resistant against the immune serum of the original strain, but Schüffner and Mochtar found that this was not the case, the daughter-cultures made from these survivors reacting in just the same way as the original ones. It is possible that these resistant forms may be the cause of persistent infections. They may multiply in the body, but of their progeny only exceptional individuals could survive, the others being destroyed by the antibodies and possibly helping to promote the formation of fresh antibodies.

Pettit (1928) has observed sera which had such a strong lytic effect on these spirochætes that it was impossible to observe any agglutination. Even after being kept in the ice-chest for 30 days the serum of one patient still dissolved the spirochætes in dilutions of 1 : 250, and it was only in dilutions of 1 : 4,000 that the other serum reactions could be observed. The conditions under which these effects are produced are not known.

The adhesion test. Brown and Davis (1927) showed that this test could be applied to the differentiation of the various types of *Leptospira*. The test consists of mixing immune serum with the specific organism in the presence of suitable particles. After a short interval a drop of the mixture is examined by means of dark-ground illumination and a positive result is indicated by the adherence of the particles to the organism (Figs. 26 and 27, facing p. 140).

It is of interest that these authors found clear distinctions between the free living water *Leptospira* (*S. biflexa*), *S. icterohæmorrhagiæ* (including

S. icteroides) and *S. hebdomadis*, respectively. But antibodies to the water *Leptospira* were found in a considerable number of stock laboratory animals such as white rats, guinea-pigs and rabbits. Out of 100 wild rats tested in this way, 11 per cent. contained antibodies against the water *Leptospira*; whilst antibodies against *S. icterohæmorrhagiæ*, never found in laboratory animals, were present in 36 per cent. of wild rats.

IMMUNITY.

Natural immunity. The natural resistance of most laboratory animals to this infection has already been mentioned (*ante*, p. 305). This resistance does not seem to depend upon any serological properties, for *S. icterohæmorrhagiæ* can survive for long periods in the serum of monkeys, rabbits, rats and mice, which are all more or less refractory to infection. On the other hand, phagocytosis of the spirochætes is extremely active in the case of all these animals, both *in vitro* and *in vivo*; and especially with the blood of rats and mice. The fact that when spirochætes are injected into rats they can persist in the kidneys without inconveniencing their host seems to depend on this tissue immunity.

Acquired immunity. This may be developed in three ways: by recovery from the infection; the administration of immune serum; or by vaccination. Human patients generally show the presence of immune-bodies in their serum towards the end of the second week of the disease. This immunity is of very long duration, for Uhlenhuth and Fromme (1918) found that the serum was still protective 22½ years after an attack of the disease. Moreover, convalescent serum may possess a very high degree of immunity, as in one case 0.01 c.cm. of a patient's serum mixed with 1 c.cm. of blood from an infected guinea-pig destroyed the virus within an hour, so that when the mixture was inoculated into a normal guinea-pig no infection resulted.

The administration of immune serum to guinea-pigs in doses of 1 to 2 c.cm. produces an immunity of short duration. If spirochætes be inoculated into the coelome of such an animal they are phagocytized within 15 minutes, whilst in the coelome of a normal guinea-pig they persist for several hours. Moreover, the peritoneal exudate of the protected animal is found to contain protective antibodies—agglutinins and lysins. Vaccination can be successfully applied in the case of guinea-pigs by the repeated inoculation of small doses of spirochætes killed either by heat or antiseptics, or by the use of cultures of low virulence. Under these conditions guinea-pigs develop an active immunity, as shown by the appearance of immune-bodies in their blood, and become resistant to the inoculation of fatal doses of the virus.

SERUM THERAPY.

The earlier workers on this disease in Japan, France and Germany all found that it was possible to produce a therapeutic serum by the inoculation of spirochætes into horses or rabbits. At first the tissues of

infected guinea-pigs were used for inoculation, but this method has been superseded by the use of cultures of the organism. Martin and Pettit (1919), for the preparation of antispirechætal serum in horses, recommend that injections of the cultures should be given every week and administered subcutaneously. The first two injections consist respectively of 1 c.cm. and 10 c.cm. of the culture and are mixed with Gram's iodine solution; the subsequent injections consist of pure cultures as rich as possible, and the dose is gradually increased up to a maximum of 100 c.cm. After 3 or 4 months the serum of the horse gradually acquires passively protective properties against the organism, but its agglutinating power is of never more than moderate value. It can be administered to man and experimental animals without producing any ill effects. Its prophylactic value can readily be demonstrated, and in the case of one horse doses of 0·0125 c.cm. were found to protect guinea-pigs against the inoculation of 1 gm. of the liver of a heavily infected guinea-pig (Griffith, 1919). This seems to be an exceptionally high degree of potency, however, for Okell, Dalling and Pugh (1925), who prepared a horse-serum against a rat strain, found that 0·4 c.cm. of serum was necessary to protect guinea-pigs against a similar dose of the virus.

This horse antiserum has been used therapeutically, both in man and dogs infected with spirochætal jaundice, and, especially if given in the early stages of the disease, produces very favourable results. Intravenous injections are said to be effective up to the fifth and sixth days of illness. Large doses of the serum are employed, up to 60 c.cm. in 24 hours, and it is said to be always preferable to make injections intravenously (Inada, Ido, Hoki, Ito and Wani, 1916). Under such conditions the spirochætes disappear from the blood within 24 hours, and the treatment promotes the development of antibodies and reduces the number of spirochætes in the organs.

The results of serum therapy in Japan seem to indicate that it has some slight effect on the course of the disease, for in one epidemic with a mortality of 30·6 per cent. in untreated patients, those receiving serum treatment showed mortalities of 23·7 per cent. after intravenous, and 17·3 per cent. after subcutaneous injections, respectively. Another epidemic with the high mortality of 57 per cent. showed a mortality of 40 per cent. after subcutaneous, and 38·5 per cent. after intravenous injections of serum (Inada, 1917).

CHEMOTHERAPY.

In view of the favourable results obtained by Uhlenhuth and Seiffert (1928, 1929) by the use of Bismuth Yatren A in the treatment of guinea-pigs infected with *S. icterohæmorrhagiæ*, this method may ultimately replace serum therapy. For human beings the authors recommend that intravenous injections of 2 c.cm. of the drug should be given every other day, but there are no records of the treatment of any human cases of the disease.

Spirochæta (Leptospira) autumnalis.

(*Synonyms* : Akiyami Type A, *Spirochæta autumnalis* A.)

A disease referred to as Akiyami or harvest sickness has been known for many years in Japan, especially in the Province of Shizuoka, near the river Oi. The epidemiology of the disease recalls that of seven-day fever, since all classes of people are liable to be attacked, not particular groups, as in the case of Weil's disease. Clinically the disease resembles a mild form of spirochætal jaundice. There is a sudden onset of fever which lasts for 5 to 8 days terminating with lysis. The patients show great fatigue, muscular pains, engorgement of the conjunctivæ, and slight enlargement of the lymphatic glands. A slight jaundice is often present, but there are no hæmorrhages or exanthem. Albuminuria is frequently present.

Kitamura and Hara (1918) were the first to prove the spirochætal origin of this disease as they were able to infect guinea-pigs by the inoculation of blood and urine from patients, and to obtain a culture of the organisms. The spirochætes obtained were found to belong to two distinct types, one of which produced fatal infections in guinea-pigs, with jaundice and hæmorrhages, whilst the other type was almost non-pathogenic towards adult guinea-pigs. This very surprising discovery was confirmed by Koshina, Shiwozawa and Kitayama (1924), who also found that two types of spirochætes occurred in patients suffering from this disease. One type, referred to as Akiyami Type A, was found to approach *S. icterohæmorrhagiæ* in its characters, but could be distinguished serologically, whilst the other variety, called Akiyami Type B, was found to resemble closely *S. hebdomadis* both in its pathogenic and serological characters. Further observations, such as those of Stéfánopoulo and Hosoya (1928), have confirmed these views, and now *S. autumnalis* Type B (= Akiyami B) is generally considered to be identical with *S. hebdomadis*. *S. autumnalis* Type A, on the other hand, can be distinguished from *S. icterohæmorrhagiæ* by its serological characters.

The two types grow together in the same cultures and are both killed by exposure to 45° C. Type B, however, is much more resistant to variations in the pH of the medium than Type A.

Guinea-pigs may readily be infected with *S. autumnalis* by either percutaneous, subcutaneous or intraperitoneal inoculation of cultures or other infected material. After a short febrile period the temperature falls and the guinea-pig shows jaundice of the sclerotic and integument. Finally hæmorrhages appear in the nasal mucosa, and the genital and anal regions, and death usually occurs 4 to 5 days after the date of inoculation. The post-mortem features closely resemble those of guinea-pigs dead of Weil's disease, but the hæmorrhages are generally more abundant in the case of infections of *S. autumnalis*. The spirochætes occur in very great numbers in the liver, but pure cultures are best obtained by collecting a few drops of heart-blood.

The infection is difficult to reproduce in the rabbit, but Stéfanopoulo and Hosoya (1928) succeeded in infecting one out of six rabbits by the intratesticular inoculation of a highly virulent culture. This rabbit died with symptoms of jaundice six days later, and spirochætes were observed in its heart-blood. Field-mice, *Microtus montebelloi*, collected in a district where the disease was prevalent, were found to be passing in their urine organisms which corresponded with Type A in their virulence.

Type B (= *S. hebdomadis*), on the other hand, rarely produces any signs of infection in guinea-pigs and never in rabbits; yet in man it produces the same type of disease as Type A.

It would seem that clinically it is almost impossible to distinguish cases of infection with *S. autumnalis* (Akiyami Type A) from cases of seven-day fever caused by *S. hebdomadis* (Akiyami Type B). It is more reasonable, therefore, to assume that the two infections occur side by side and have been confused, than to accept the original view that the same disease might be caused by two distinct types of spirochætes. Consequently the cause of Akiyami disease is referred to simply as *S. autumnalis*, the so-called Type B becoming a synonym of *S. hebdomadis*.

***Spirochæta (Leptospira) hebdomadis* Ido, Ito and Wani, 1918.**

(*Synonym* : Akiyami Type B.)

This organism, isolated by Ido, Ito and Wani (1918) in Japan from cases of seven-day fever, known locally as Nanukayami, is distinguished entirely by its immunological reactions. The spirochæte is present in the blood of patients during the first week and can sometimes be found by direct microscopical examination. Usually, however, it is necessary to make cultures of the blood, or inoculations into guinea-pigs. After the first week the spirochætes seem to enter the kidneys and are present in the urine from the eighth to the fortieth days. The spirochæte is pathogenic only to young guinea-pigs, in which it produces hypertrophy of the lymphatic glands and a slight hæmorrhagic jaundice, with a mortality of about 60 per cent. Jaundice was noted in 17·4 per cent. and nasal hæmorrhage in 4·4 per cent. compared with guinea-pigs inoculated with Weil's disease in which the mortality is 100 per cent., jaundice 99·9 per cent., and nasal hæmorrhage 78 per cent.

The natural reservoir of the disease is said to be the field-mouse, *Microtus montebelloi*, and about 3 per cent. of these animals collected in an endemic region were found to contain the spirochætes in their urine. The disease may be transmitted by the agency of these rodents, for the patients are generally agricultural labourers and foresters. The onset is sudden, accompanied by fever, prostration, injection of the conjunctiva, muscular pains, digestive troubles and enlargement of the lymphatic glands. Albuminuria is present and the blood shows a marked leucocytosis. The disease only lasts for about one week and is never fatal.

Spirochæta (Leptospira) pyrogenes Vervoort, 1923.*(Synonym : Spirochæta febrilis.)*

This organism was first described by Vervoort (1923^a) as occurring in the blood of patients suffering from dengue-like fevers in Deli, Sumatra. Cultures of the spirochætes were obtained from the blood and found to resemble closely *S. icterohæmorrhagiæ* in their general appearance and behaviour. Guinea-pigs were infected both by the inoculation of blood from fever patients and by cultures. The guinea-pigs generally died as a result of the infection and showed jaundice and hæmorrhages, whilst spirochætes were present in their blood and urine. Subsequently, this strain of spirochætes was found to be serologically distinct from other known strains (Bonne, 1924), and therefore the name *pyrogenes* has been retained.

Fletcher (1928) made an extensive study of the leptospiral infections in the Federated Malay States and found that half of those examined belonged to this type. It is probable, therefore, that *S. pyrogenes* is widespread in Malay and the Dutch East Indies, where it produces fevers of short duration. The most constant symptoms observed were sudden onset and great prostration, and especially the agonizing muscular pain and tenderness. The presence of casts and albumin in the urine, although only in small quantity, serves to distinguish the disease from dengue, with which it has probably been confused in the past. Pronounced redness of the eyes is another important symptom. Jaundice, on the other hand, is often absent.

It should be noted, however, that Fletcher isolated six serologically distinct groups of spirochætes from patients in Malaya, all showing very similar clinical symptoms, therefore there seems to be little, if any, difference between *S. pyrogenes* and other strains of pathogenic *Leptospira* as regards the effects produced in human beings.

Doubtful Forms.

SPIROCHÆTA (LEPTOSPIRA) HÆMOGLOBINURIÆ SCHÜFFNER, 1918,
SPIROCHÆTA BILIOHÆMOGLOBINURIÆ BLANCHARD AND LEFROU, 1922.

Organisms morphologically identical with *S. icterohæmorrhagiæ* were found by Schüffner (1918) in the blood of a Javanese patient who was admitted to hospital suffering from an attack of blackwater fever. A few tertian malaria parasites were found in his blood, and in addition spirochætes of the leptospiral type. Two guinea-pigs were inoculated, but with negative results.

Noc (1920) records that in Dakar, Dr. Esquier found spirochætes, together with malaria parasites, in the liver of a fatal case of blackwater. The organisms were said to be thicker than the spirochætes of Weil's disease. Finally, Blanchard and Lefrou (1922¹), in a series of papers, recorded the discovery of spirochætes in the blood of three blackwater-fever patients at Brazzaville. The organisms were said to be extremely

rare in the blood and were only discovered by a process of triple centrifuging. Ten c.cm. of blood were taken from the vein and mixed with 1 c.cm. of 20 per cent. citrate solution in a centrifuge tube. The mixture was centrifuged at 1,500 revolutions to the minute until most of the cells had been deposited at the bottom of the tube. The supernatant fluid was then drawn off and again centrifuged until the remaining cells had come down. Finally, the fluid portion was again removed and centrifuged at high speed for a longer period. Spirochætes were found in the deposit in this third tube. They are stated to be 6 to 9 μ in length, 0.1 to 0.2 μ in width, and in their general appearance resemble the spirochætes of infective jaundice.

The organisms were successfully inoculated into guinea-pigs, but it was only on the third passage that pathological symptoms were observed. Jaundice, which is always a prominent feature of guinea-pigs infected with spirochætal jaundice, was not observed and consequently it is assumed that the organism was distinct.

It must be admitted that the evidence in support of the ætiological significance of these findings is most unsatisfactory. Blanchard and Lefrou (1926), in a subsequent communication, attempted to distinguish clinically between cases of blackwater in which spirochætes could be found and those in which they were absent. Judging from the authors' descriptions there seem to be no constant differences, and J. G. Thomson (1924) in Rhodesia, Low and Duncan (1923) in London, and many subsequent investigators have entirely failed to discover these organisms in undoubted cases of blackwater. It seems most probable, therefore, that *Spirochæta biliohæmoglobinuriæ* is only a secondary infection of no ætiological significance in blackwater fever.

SPIROCHÆTA (LEPTOSPIRA) SP.

A spirochæte closely resembling *S. icterohæmorrhagiæ* was also obtained at Brazzaville by triple centrifuging of the blood of Congo natives suffering from infective jaundice. Blanchard, Lefrou and Laigret (1923) were able to infect guinea-pigs with this spirochæte and the animals died with jaundice and hæmorrhages and other symptoms generally resembling Weil's disease. During four passages the guinea-pigs died of the disease, but at the fifth there was no sign of illness and the virus was lost.

The peculiar property of this spirochæte was its power of surviving in bed bugs, *Cimex lectularius*, in which it differs from ordinary *S. icterohæmorrhagiæ*. According to Blanchard and Lefrou (1922²) bed bugs were still infective to guinea-pigs 38 days after being fed on an infected animal. In addition, the authors consider that there is strong presumptive evidence in support of the view that it is conveyed by bed bugs from one human being to another. This may be the case, but much better evidence is necessary to justify the conclusion that the epidemic jaundice of the Congo is caused by a spirochæte distinct from *S. icterohæmorrhagiæ*. Moreover, the record of this spirochæte in the same area as that from

which the same authors found similar organisms in blackwater fever suggests the occurrence in the Congo of widespread spirochætal infections, probably associated with jaundice. Similar infections have been found in Malaya (Fletcher, 1928) and Java (Vervoort, 1923¹), and it is likely that further studies will result in the known distribution of these organisms being considerably extended.

SPIROCHÆTA (LEPTOSPIRA) PETTITI FIESSINGER, 1918.

A spirochæte, morphologically identical with *S. icterohæmorrhagiæ*, was first observed by Manine, Cristau and Plazy (1917) in the urine of a series of patients at the marine hospital of Lorient. The same spirochæte was also studied by Pettit, who showed that it was distinct from the causative organism of spirochætal jaundice. The following year Fiessinger (1918) named it *S. pettiti*, and since then what seems to be the same organism has been repeatedly found in the urine. According to Pettit (1928) the morphological characters are insufficient to distinguish it, but compared with *S. icterohæmorrhagiæ* it is said to be longer and finer, with its spires looser and much more irregular; its length is 6 to 15 μ .

All efforts to infect laboratory animals, such as guinea-pigs, &c., have given negative results, and hitherto attempts at culture have also failed.

In man various symptoms have been attributed to its presence, but the only constant feature seems to be some degree of fever. The appearance of spirochætes in the urine is intermittent, but when present they are generally in considerable numbers. They appear in the urine either during the rise in temperature or when it ends. The serum of patients infected with *S. pettiti* has no effect on *S. icterohæmorrhagiæ*.

Whether this organism has any pathogenic action seems very doubtful, and further observations are necessary to determine its nature.

SPIROCHÆTA (LEPTOSPIRA) INTERROGANS STIMSON, 1909.

This organism was found by Stimson (1909) in the kidney of a yellow-fever patient. The specimen had been stained by Levaditi's method, and the author gave the following description of the organism found in the kidney: 'Colour, opaque black, in sharp contrast to the surrounding tissues, general appearance strongly suggesting a *spirochæte*; often irregularly curved, but some individuals having a regular series of alternate curves, no other indication of segments being observed; one or both extremities often bent back in the form of a hook; entire length variable up to 14 μ or more; width estimated at $\frac{1}{4}\mu$; length of short curves $1\frac{1}{2}$ to 2 μ . It was confined to the cells and lumina of the tubules of the kidney, none being observed in the blood-vessels, glomeruli, or interstitial tissue'. For convenience the name (? *Spirochæta*) *interrogans* was suggested.

It seems probable that this was a case of Weil's disease, but in the absence of any further information on this organism the evidence as to its nature is so uncertain that it seems undesirable to adopt the name

S. interrogans in place of *S. icterohæmorrhagiæ*. Should, however, the views advanced by Zuelzer, as to the specific unity of all leptospiral forms, become generally adopted, the priority of *S. interrogans* as a name for them might have to be recognized.

SPIROCHÆTA (LEPTOSPIRA) COUVYI J. GOMES DE FARIA, 1924.

This name has been applied to a spirochæte first recorded by Couvy (1921) at Beyrouth, as occurring in the blood of patients suffering from dengue fever. According to this author the spirochætes are extremely fine with pointed extremities and only show two or three spiral turns. They were only seen five or six times and then only during the incubation period of the disease, one or two hours before the onset of fever. Subsequently, Couvy (1922) again recorded the finding of spirochætes in cases of so-called dengue at Beyrouth. The organisms were observed not only before the onset of fever but during 3 to 48 hours after; they were always very scanty. The inoculation of the blood of these patients into rabbits gave rise to a fever, and spirochætes were found in these animals at the time of the onset or relapse. Three passages were made in rabbits without alternating the virus. During this 1921 epidemic in the Lebanon only *Phlebotomus* were found, and the temperature chart given by the author might very well illustrate sandfly fever, for true dengue is now known to be transmitted by *Aedes*. Attempts to transmit the disease by feeding *Phlebotomus* on infected rabbits and subsequently on healthy ones gave negative results, but the crushed contents of these flies when inoculated intraperitoneally into rabbits produced fever, and spirochætes were recovered from the blood.

Gomes de Faria (1924) recorded a similar organism from the blood of dengue patients in Rio de Janeiro and named it *Leptospira couvyi*.

What is possibly the same organism was found independently by Whittingham (1922) in the blood of sandfly-fever cases in Malta. A spirochæte resembling *S. icterohæmorrhagiæ* was isolated on the first day of fever by direct culture of the blood of six cases of the disease, but when the cultures were inoculated into non-immunes they failed to reproduce the infection. Moreover, the organism was not pathogenic to guinea-pigs, although most of the animals showed a rise of temperature of about 2° F. for the first 48 hours after being inoculated.

Up to the present the significance of these findings has not been made clear, but the frequency with which spirochætes, especially of the *Leptospira* type, have been found in the blood of patients suffering from various types of fevers suggests that they may have some pathological significance. The most reasonable hypothesis is that mild cases of spirochætal jaundice, sometimes only producing febrile symptoms, are very widespread, and having no very definite clinical manifestations are liable to be confused with other diseases, such as dengue, sandfly fever, yellow fever, &c. Recent observations have shown that cases of Weil's disease have been diagnosed as yellow fever, and it is not unreasonable to

suppose that other diseases may also have been confused with it, but until better evidence is available the spirochætal nature of dengue and sandfly fever must be regarded as very doubtful.

REFERENCES.

- BAERMANN, G. & ZUELZER, M., 1928, *Zbl. Bakt.*, Abt. I, Orig., **105**, 345.
 BASILEWSKY, B. G., 1930, *Zbl. Bakt.*, Abt. I, Orig., **116**, 173.
 BESSEMAN, A. & THIRY, U., 1928, *C.R. Soc. Biol.*, Paris, **90**, 1881; 1930, *ibid.*, **103**, 519.
 BLANCHARD, M. & LEFROU, G., 1922¹, *Bull. Soc. Path. exotique*, **15**, 699; 1922², *C.R. Acad. Sci.*, Paris, **179**, 602; 1926, *Bull. Soc. Path. exotique*, **19**, 345.
 BLANCHARD, M., LEFROU, G. & LAIGRET, J., 1923, *Bull. Soc. Path. exotique*, **16**, 184.
 BONNE, C., 1924, *Bull. Soc. Path. exotique*, **17**, 623.
 BROWN, H. C. & DAVIS, L. J., 1927, *Brit. J. Exp. Path.*, **8**, 397.
 BUCHANAN, G., 1924, *Brit. Med. J.*, ii, 990; 1927, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 113.
 COSTA, S. & TROISIER, J., 1916, *C.R. Soc. Biol.*, Paris, **79**, 1038.
 COURMONT, J. & DURAND, P., 1917, *C.R. Soc. Biol.*, Paris, **80**, 275.
 COUVY, L., 1921, *Bull. Soc. Path. exotique*, **14**, 198, 447; 1922, *Ann. Inst. Pasteur*, **36**, 851.
 DAMON, S. R. & HAMPIL, B., 1929, *J. Bact.*, **18**, 343.
 DUNKIN, G. W. & LAIDLAW, P. P., 1926, *Rep. Med. Res. Coun.*, 1924-5, p. 31.
 ETCHGOIN, E., 1924, *C.R. Soc. Biol.*, Paris, **90**, 682; **91**, 1005.
 FIESSINGER, N., 1918, *Ann. Méd.*, **5**, 156.
 FLETCHER, W., 1928, *Trans. Soc. Trop. Med. Hyg.*, Lond., **21**, 265.
 FOULERTON, A. G. R., 1919, *J. Comp. Path.*, **32**, 182.
 FROMME, 1919, *Berl. tierärztl. Wschr.*, **35**, 33.
 GOMES DE FARIA, J., 1924, *C.R. Soc. Biol.*, Paris, **90**, 55.
 GRIFFITH, A. S., 1919, *J. Hyg.*, Camb., **18**, 59.
 HINDLE, E., 1925, *Brit. Med. J.*, ii, 57.
 HÜBENER, H. & REITER, H., 1915, *Deuts. med. Wschr.*, **41**, 1275.
 IDO, Y., ITO, H. & WANI, H., 1918, *J. Exp. Med.*, **28**, 435.
 INADA, R., 1917, *Tokyo Med. News*, 1917, 24.
 INADA, R. & IDO, Y., 1915, *Tokyo Ijishinshi*, No. 1908.
 INADA, R., IDO, Y., HOKI, R., ITO, H. & WANI, H., 1916, *J. Exp. Med.*, **24**, 485.
 INADA, R., IDO, Y., HOKI, R., KANEKO, R. & ITO, H., 1916, *J. Exp. Med.*, **23**, 377.
 KATHE, 1928, *Zbl. Bakt.*, Abt. I, Orig., **109**, 284.
 KITAMURA, S. & HARA, S., 1918, cited from *Trop. Dis. Bull.*, 1920, **16**, 169.
 KLARENBECK, A., 1928, *Tijdschr. Diergeneesk.*, **55**, 227.
 KÖRNER, 1925, *Deuts. med. Wschr.*, **51**, 772.
 KOSHINA, Y., SHIWOZAWA, S. & KITAYAMA, K., 1924, cited from *Japan. Med. Wld.*, **4**, 268.
 KRIVÁČEK, O., 1924, *Z. Hyg. InfektKr.*, **103**, 529.
 KRUMBEIN, R. & FRIELING, B., 1916, *Deuts. med. Wschr.*, **42**, 564.
 LOW, G. C. & DUNCAN, J. T., 1923, *Trans. Soc. Trop. Med. Hyg.*, Lond., **17**, 201.
 LUKÈS, J., 1924, *Ann. Inst. Pasteur*, **38**, 523.
 LYON, D. M., 1925, *Lancet*, Lond., i, 504.
 MANINE, CRISTAU & PLAZY, 1917, *C.R. Soc. Biol.*, Paris, **80**, 531.
 MANSON-BAHR, P., 1922, *Lancet*, Lond., ii, 1056.
 MARTIN, L. & PETTIT, A., 1919, *Monog. Inst. Pasteur*, Masson et Cie, Paris.
 MIDDLETON, A. D., 1929, *J. Hyg.*, Camb., **29**, 219.
 MONTI, A., 1916, *Boll. Soc. med. chir.*, Pavia, Nos. 3 and 4.
 NICOLLE, C. & LEBAILLY, C., 1918, *C.R. Soc. Biol.*, Paris, **81**, 351.
 NOC, F., 1920, *Bull. Soc. Path. exotique*, **13**, 672.
 NOGUCHI, H. & LINDENBERG, A., 1925, *Amer. J. Trop. Med.*, **5**, 63.
 OBA, S., 1923, *Aichi J. Exp. Med.*, **1**, 1.
 OKELL, C. C., DALLING, T. & PUGH, L. P., 1925, *Vet. J.*, **81**, 3.
 OKELL, C. C. & HOUSTON, A., 1926, *20th Rep. Metrop. Wat. Bd.*, London, 1925-6, 43-51.

- PETTIT, A., 1928, *Contribution à l'étude des Spirochètidés*, Chez l'auteur, 70 rue Jullien, Vanves (Seine).
- SANI, L., 1919, *Ann. Igi.*, **29**, 76.
- SARDJITO, M. & ZUELZER, M., 1929, *Zbl. Bakt.*, Abt. I, Orig., **110**, 180.
- SCHÜFFNER, W., 1918, *Geneesk. Tijdschr. Ned.-Ind*, **58**, 352.
- SCHÜFFNER, W. & MOCHTAR, A., 1927, *Konink. Akad. Wetensch. Amsterdam*, **30**, 1.
- STÉFANOPOULO, G. J. & HOSOYA, S., 1928, *C. R. Soc. Biol.*, Paris, **98**, 1317.
- STEVENSON, A. C., 1922, *Amer. J. Trop. Med.*, **2**, 77.
- STIMSON, A. M., 1909, *Trans. Soc. Trop. Med. Hyg.*, Lond., **3**, 56.
- THOMSON, J. G., 1924, *Proc. R. Soc. Med.* (Sect. Trop. Dis. & Parasitol), **17**, 47.
- TOHYAMA, Y., 1927, *Sci. Rep. Govt. Inst. Infect. Dis. Tokyo*, 1927, **6**, 555.
- UHLENHUTH, P. & FROMME, W., 1915, *Med. Klin.*, **11**, 1202, 1264, 1296 and 1375; 1918, *Deuts. med. Wschr.*, **44**, 705; 1930, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **7**, 487.
- UHLENHUTH, P. & SEIFFERT, W., 1928, *Med. Klinik.*, **24**, 584; 1929, *Zbl. Bakt.*, Abt. I, Orig., **114**, 241.
- UHLENHUTH, P. & ZUELZER, M., 1921, *Zbl. Bakt.*, Abt. I, Orig., **85**, Beiheft, 141.*
- VERVOORT, H., 1923¹, *Geneesk. Tijdschr. Ned.-Ind.*, **63**, 800, 1923², *Rep. Far East. Assoc. Trop. Med.*, 1923, p. 683, J. Bale Sons & Danielsson, London.
- VINZENT, R., 1926, *C.R. Soc. Biol.*, Paris, **95**, 1472.
- WENYON, C. M., 1926, *Protozoology*, Baillière, Tindall & Cox, London.
- WHITTINGHAM, H. E., 1922, *Proc. R. Soc. Med.* (War Section), **16**, 1.
- WILBERT, R. & DELORME, M., 1927, *Ann. Inst. Pasteur*, **41**, 1139.
- WOLBACH, S. B. & BINGER, C. A. L., 1914, *J. Med. Res.*, **30**, 23.
- ZUELZER, M., 1922, *Zbl. Bakt.*, Abt. I, Orig., **89**, 171.

CHAPTER X. YAWS.

BY ALEXANDER DAWSON
(LIEUT.-COL., ROYAL ARMY MEDICAL CORPS).

History.

UP to 1905 various micro-organisms, bacilli, cocci and yeasts were suggested as being the cause of yaws, but no satisfactory evidence as to their action was produced. Castellani (1905¹) noticed what he considered to be spirilla in Leishman-stained smears from scrapings of yaws ulcers. On Schaudinn's announcement of the finding of *Spirochæta pallida* in syphilitic lesions, Castellani (1905²) continued his investigations of yaws and found spiral organisms, some of which were indistinguishable from *S. pallida*. For these he suggested in turns the names *Spirochæta pertenuis* and *Treponema pertenuis*. Wenyon (1926), however, considers that the correct name is that which was first given to the organism, namely *Spirochæta pertenuis*. Castellani's discovery of *S. pertenuis* was soon verified by many workers, and that organism is now regarded as being the cause of yaws.

General Description of the Causative Organism and its Relation to Other Organisms.

For practical purposes *S. pertenuis* may be regarded as being morphologically indistinguishable from *S. pallida* and from *S. cuniculi*. Minor differences have been described by workers at various times, but most agree with the view of Castellani and Chalmers (1919¹) that *S. pallida* and *S. pertenuis* can be distinguished from one another only by biological tests. The interrelationship of these three spirochætes is not definitely known, but, broadly speaking, *S. pallida* and *S. pertenuis* appear to be more closely related to one another than are both to *S. cuniculi*, which is sharply differentiated from the others by being infective only for rabbits and in these producing more superficial lesions. *S. pertenuis*, though infective for the same animal species as *S. pallida*, appears to be generally less invasive.

Staining and Cultivation.

S. pertenuis does not take ordinary stains readily, but, in smears, it can be stained by fixing with a volume of Leishman's stain for five minutes, then adding two volumes of neutral distilled water and, after half an hour, washing off with distilled water. This was the method originally employed by Castellani. All the other methods used for demonstrating *S. pallida* are suitable for *S. pertenuis*.

The same difficulties have been found in the cultivation of *S. pertenuis* as occur in the case of *S. pallida*, and no work of practical importance has been done with cultures. By methods similar to those employed in the

cultivation of *S. pallida*, Noguchi (1911), Baermann and Heinemann (1913) and others have claimed success in obtaining cultures of *S. pertenuis*, but animals have not been successfully inoculated with the alleged cultures, and Ford (1927) considers that the organism has not been cultivated.

Distribution and Persistence.

VIABILITY.

Yasuyama (1928) cites the finding of Ashburn and Craig that *S. pertenuis* remained active for several days in yaws material kept in capillary tubes. Yasuyama tested the duration of infectivity of yaws material, kept under certain conditions of time, temperature and suspension medium, after its removal from experimentally infected monkeys. He found that, at a room temperature of 28.5° C., motility ceased in from 30 minutes to 2 hours, there being no apparent difference whether the spirochaetes were suspended in the experimental animal's serum, in horse-serum, or in physiological saline solution. In tests of virulence he found that scrapings of yaws lesions, suspended in saline solution, were infective up to 30 minutes at 28.5° C. and up to an hour at 37° C., but after 30 minutes at 5° C. the material was non-virulent. Serum from yaws lesions remained infective up to 2 hours at room temperature (28.5° C.), but was non-infective at the end of 30 minutes at 0° C. In another experiment, Yasuyama allowed flies to feed on yaws lesions; emulsions of the flies, made up to 30 minutes after feeding, were infective for monkeys, but inoculations with emulsions made after an hour were unsuccessful, although they contained *S. pertenuis*. The infectivity of *S. pertenuis* seems, therefore, to disappear very quickly after removal from the susceptible animal.

DISTRIBUTION IN ANIMALS.

There is no evidence that *S. pertenuis* occurs naturally in animals, although Modder (1907), in suggesting that yaws may be transmitted by ticks, stated that in 1906 he came across two cattle in Ceylon which 'appeared to be suffering from yaws', and a boy attending to them was suffering from yaws which seemed to have originated in a tick bite. Modder also said he removed ticks from a bull 'suffering from yaws', placed them in distilled water and, on staining the deposit from the water, he found spirochaetes. Modder, however, gives no description of the disease in the animals, or of the spirochaetes he saw. There appears to be no confirmation of these observations by other workers.

DISTRIBUTION IN MAN.

Castellani (1907¹), Von dem Borne (1907) and many others have shown that *S. pertenuis* can be demonstrated in almost 100 per cent. of intact yaws lesions, in a fairly high percentage of ulcerated lesions, but rarely in old lesions.

In one of his earliest cases Castellani (1905³) found *S. pertenuis* in smears made from a gland excised from the neck of a yaws patient, and he reported later (1907¹) that he had found *S. pertenuis* in lymphatic glands in 6 of 11 cases and in the spleen juice in 3 of 5 cases examined. He failed to find the spirochæte in the circulating blood in 20 cases examined, although he succeeded in infecting a macacus monkey by rubbing into its scarified eyebrow 1 c.cm. of blood taken from a vein of a yaws patient. He failed to find the spirochæte in the cerebrospinal fluid of individuals suffering from yaws, and attempts to infect monkeys by means of spinal fluid were unsuccessful.

Schüffner (1907), using Bertarelli and Volpino's silver method, demonstrated *S. pertenuis* in sections and found them to be limited to the epidermis. This accords with the histological findings of Macleod (1901), who described the initial lesions of yaws as occurring in the superficial layers of the corium. Goodpasture (1923) showed that *S. pertenuis*, in addition to being abundant in the thickened epidermis, is also present in the perivascular connective tissue of the papillæ.

Pathogenic Action.

SUSCEPTIBILITY OF EXPERIMENTAL ANIMALS.

Shortly after the discovery of *S. pertenuis* in yaws lesions it was found that the disease could be transmitted to experimental animals. Neisser, Baermann and Halberstadter (1906) succeeded in infecting monkeys with human yaws; in gibbons and anthropoid apes the incubation period was from 13 to 14 days, while in lower monkeys it varied from 22 to 91 days. They also succeeded in transferring the disease from one monkey to another.

Castellani (1907²), after a few failures, was similarly successful with *Semnopithecus* and *Macacus* monkeys by rubbing scrapings from unbroken yaws lesions into the scarified eyebrows. The incubation period varied from 9 to 90 days. Fluid from yaws lesions after filtration through a Berkefeld filter, 12A, failed to infect monkeys. Castellani described the yaws lesion which appeared at the site of inoculation on the monkeys as being a flattened papule surrounded by an infiltrated zone. The papule rapidly enlarged, became moist and covered with a thick crust, on the removal of which a granulating surface was seen. Histologically, there was marked proliferation of the interpapillary processes and a cellular infiltration consisting of plasma cells, some extravasated polymorphonuclear leucocytes, small mononuclear cells, connective tissue cells and a few mast cells.

For many years workers in the Philippine Islands have used Philippine monkeys (*Cynomologus philippinensis*) as the experimental animal in their work on yaws. Schöbl (1928¹), in an extensive series of experiments on Philippine monkeys, found that after inoculation with yaws material there developed in, on an average, 26 days a papule which gradually spread,

becoming cracked and covered with a softish crust. In about 21 days the lesion usually healed, and, in a few cases, exacerbations occurred on skin previously affected. Generalization of skin lesions did not occur on primary infection of monkeys, but Schöbl obtained this after superinfection and reinfection, the later lesions being clinically and histologically similar to the generalized lesions in man.

Transmission to rabbits was first reported by Nichols (1910), the inoculation being with serum from a yaw on a monkey. The material was injected into the rabbit's testicle. In 24 days the rabbit's testicle became swollen and firm, and serum extracted on several occasions showed a few spirochætes. A monkey was infected from the rabbit's testicle, and from this monkey 3 out of 8 rabbits were successfully inoculated. The strain was carried on from this last series of rabbits. Nichols considered the change in the rabbit's testicles to be an interstitial orchitis with tubular disintegration and a cellular infiltration, of small round cells and endothelial cells, in œdematous connective tissue. In sections stained by Levaditi's method Nichols found spirochætes in large numbers in and around the tubules and in interstices of the connective tissue. The ability of the spirochæte to spread to other parts of the body was demonstrated by the fact that, in some cases, the non-inoculated testicle became swollen, and spirochætes were found in it.

Pearce and Brown (1925), working with two strains of *S. pertenuis* transmitted through rabbits, one for two and a half years and the other for three years, found that a granular or finely nodular type of periorchitis, with or without involvement of the tunica vaginalis, was produced on testicular inoculation and that this was quite different from the lesions they had described in syphilitic infections of rabbits' testicles. Nichols (1925), judging by the action of the spirochætes in rabbits, considered that there are differences in various strains of *S. pertenuis* and that the Philippine strain was midway between the West Indian strain and *S. pallida*.

Transmission to mice was successfully carried out by Schlossberger (1927), working with a strain of *S. pertenuis* obtained from Nichols. Pieces of infected tissue containing many spirochætes were implanted in skin pockets on the backs of the mice, and, although no sign of disease appeared in the inoculated animals, infection was proved by injecting various tissues and organs from them into rabbits' scrotal skin and reproducing the disease. Brain emulsion from one mouse removed two months after yaws inoculation produced the disease in the rabbit, while lymphatic gland and spleen emulsion from the same animal failed. From another mouse, six months after inoculation, brain emulsion and a combined emulsion of spleen and lymph gland gave successful results. In a third experiment, nine months after implantation of yaws, an emulsion of gland and spleen was successful, but brain emulsion and blood failed. Schlossberger drew attention to the first mouse, in which brain emulsion was successful, and pointed out that if the more commonly used spleen

and lymphatic gland tissues only had been employed, the mouse would have been considered non-infected.

PATHOLOGY IN MAN.

Method of Transference.

It is considered that the disease is generally transferred by direct contact. Castellani and Chalmers (1919²) noted that in the cases they saw in Ceylon the primary lesion was almost always extragenital and might be found on an old ulceration, on an insect bite, on an itch pustule, on a wound or a vaccination mark, the smallest abrasion being sufficient for entry of the virus. Their women patients usually showed the primary lesion in the region of the nipple, and in some cases on the trunk above the hip, positions which are exposed to the contact of children. The disease is commonest in children and young people, but rare in Europeans and better-class natives in the countries in which it is prevalent, insanitary conditions favouring transmission. Hanschell (1928) stated that in Barbados yaws was at one time common but had not occurred for generations, though syphilis is prevalent, and he suggested that yaws disappeared when the negroes took to wearing clothes.

Yaws is found to be more prevalent in country districts than in towns, which is in contrast to the occurrence of syphilis. The disease has been transmitted from man to man by inoculating material from yaws lesions. Manson-Bahr (1928) stated that Fijian women have been in the habit of inoculating their infant children with yaws to prevent their being attacked later in life. Paulet (1848) inoculated secretion from lesions on yaws patients into 14 negroes; primary lesions developed in 12 of them in from 14 to 28 days; in 2 no primary lesion developed, but all eventually showed generalized eruptions of yaws. Charlouis (1881) also successfully inoculated men with material from yaws lesions, and noted that injection with blood from dry tubercles in retrogression was unsuccessful.

Transmission by flies is a possibility and has been carried out experimentally by Castellani and by Yasuyama. Castellani (1907²) allowed house-flies, caught in his laboratory in Ceylon, to feed on yaws patients and on yaws material, placed them on the scarified eyebrows of monkeys and succeeded in infecting 3 of 10 monkeys employed. The experiments of Yasuyama with emulsions of flies which had fed on yaws lesions have been mentioned (p. 321).

Hereditary transmission has not been observed.

Modes of Spread and Distribution in the Body.

It is presumed that the spirochæte is distributed in the body by the blood-stream. Castellani (1907²) showed that the disease could be transferred from man to an experimental animal by inoculation with the circulating blood. The secondary yaws lesions may appear on the skin in any part of the body, and *S. pertenuis* is demonstrable in them. The spirochæte is also found in the lymphatic system, in the lymphatic

glands, which become enlarged. The spleen is frequently enlarged, and Castellani (1907²) found *S. pertenuis* in spleen juice, with which he succeeded in infecting two monkeys.

Pathological Results in Man.

Descriptions of the histological changes in yaws have been given by many authors. Those of Charlouis, in 1881, and of Nichols, in 1894, are summarized by Macleod (1901), who also gives a detailed description of his own findings. Macleod noted that, in the early squamous stage, there is dilatation of the papillary and subpapillary blood-vessels of the corium, with a marked extravasation of leucocytes around the vessels, the leucocytes passing to the lymph spaces between the fibrous bundles and eventually reaching the epiderm; endothelial proliferation and increase of the blood-vessels were not seen, in contrast to the changes in syphilitic lesions. Plasma cell infiltration was seen near blood-vessels, but not connected with them. Early changes were noted in the epiderm, interpapillary pegs became elongated and thickened, and frequently the interepithelial spaces appeared widened and œdematous. The overlying stratum granulosum was seen to be defective, with imperfect cornification and a tendency to lamellar formation. In later lesions, where the papular stage was replacing the squamous, Macleod found the above changes more marked, and the plasma cells were in diffuse sheets and more clustered round the blood-vessels. Macleod considers that the chief histological differences between yaws and syphilis are the following:

In yaws the plasma cells of the cellular infiltration are less definitely arranged in rows or clusters around the blood-vessels than they are in syphilis; rarefaction of collagen is more marked than in syphilis; there is no colloidal degeneration such as occurs in syphilitic gummata; in yaws there are no distinct proliferative changes in the walls or endothelium of the blood-vessels; in the epidermis in yaws there is marked proliferation and down-growth, with great thickening, of the horny layer, which is unusual in syphilis. The yaws lesions thus appear to be more like those produced in rabbits by *S. cuniculi*.

Until recently the lesions of yaws have been considered to be usually limited to the skin and, occasionally, bone, but Stitt (1929) states that Harper reported involvement of the circulatory systems in Fijians and that Choisser, in 526 autopsies of cases with histories of yaws infection, in Haiti, claims to have found many visceral and circulatory system lesions similar to those of syphilis. Such claims require further investigation.

Evidence of Responsibility of the Organism for the Disease.

S. pertenuis is practically always demonstrable in yaws lesions, and, as already mentioned, material from these lesions, uncontaminated by other organisms, when inoculated into susceptible animals under suitable conditions, produces yaws. Further, the organs of mice infected with yaws material are infective for rabbits.

Evidence of Responsibility for Accessory Diseases.

Gangosa, or rhinopharyngitis mutilans, which until recently was regarded as being due to leprosy, to tubercle, to syphilis, and to yaws, is now generally regarded as a sequel of yaws. Schöbl (1928¹) describes the development of gangosa in Philippine monkeys experimentally infected with yaws. He found that yaws lesions occasionally spread from the face to the mucous membrane of the nose, and in one of these cases he superinfected the animal on the eyebrow, with the result that the lesion of the mucous membrane increased considerably and developed into an extensive gangosa. In another case he reinfected the monkey on an ala nasi; a deep ulcerating lesion developed, penetrating inwards, as well as along the skin and causing great destruction of tissue. Schöbl considers that an allergic state is necessary for the development of gangosa. Kindelberger's finding that the luetin reaction was positive in a large proportion of gangosa cases (see p. 328) gives no assistance in deciding the ætiology of the condition. Rossiter (1912) reported a case resembling gangosa, in which he stated he found *S. pertenuis* in smears made from the lesions, but his evidence is not convincing.

Goundou is another condition usually attributed to yaws. Manson-Bahr (1929) states that it is supposed to be a systematized hypertrophic osteitis arising from yaws, but Sharpe (1928) described a case, previously exhibited by Davis, in a syphilitic in England, who had never suffered from yaws, and Balfour (1928) stated that, on the boat between Mauritius and Bombay, he found a horse with goundou.

Juxta-articular nodes are suspected to be a sequel of yaws.

Resistance.

Under natural conditions yaws is confined to man, and, although practically only natives of many tropical and subtropical countries are affected, there is no evidence of racial immunity as, in these countries, the disease has been found in Europeans.

Yaws, in contrast to syphilis, is much more common in country districts than in towns, and its prevalence is much greater in low-lying areas than at high altitudes, although it is by no means rare even at a height of 6,000 feet, in Kenya and other countries. Lopez-Rizal and Sellards (1926) found the disease in the Philippine Islands at heights of 1,800 to 6,000 feet, in an isolated race of people, the type of disease being unusual in that the cutaneous lesions were limited to the muco-cutaneous junctions of the mouth, nose, anus and genitalia in 90 per cent. of the cases, although generalization of the disease was indicated by enlargement of the inguinal, epitrochlear, axillary, posterior cervical and auricular lymphatic glands. Lopez-Rizal and Sellards suggested that a modified strain of *S. pertenuis*, of low dermatropic affinity, may exist in these people, but they were unable to transfer any of the patients to the lower parts of the island to observe if the type of disease changed. Ramsay

(1925), however, had observed that patients in the hills of Assam suffered from a mild type of yaws, but, on coming down to the plains, they developed the ordinary florid type of lesion. Schöbl (1928¹) states that, in experimental work on monkeys in the Philippine Islands, he found that a strain obtained from a patient near Manila produced lesions of higher infectivity for monkeys than did one obtained from a patient in the hills.

Practical Diagnosis.

DETECTION OF THE ORGANISM.

Examination, by the dark-ground method or by Leishman's staining, of serum from unbroken yaws papules practically always shows the presence of *S. pertenuis*. Smears from ulcerated lesions may or may not show the spirochæte, but, if present, it is usually in small numbers and tends to be hidden by large numbers of secondary spirochætes and other micro-organisms. As already mentioned (p. 322), *S. pertenuis* may be demonstrated in the enlarged lymphatic glands of yaws patients.

INDIRECT DIAGNOSIS.

The Wassermann reaction and various flocculation tests employed in the diagnosis of syphilis have been found to give positive results in yaws.

The Wassermann Reaction.

Moss and Bigelow (1922), working in the Dominican Republic, tested the Wassermann reaction in a series of 91 cases of yaws and found it to be positive in 91 per cent. of the cases. Of the negative cases, three were in the late secondary stage, two were in the tertiary stage, and the others gave a history but had no sign of yaws. Baermann (1927¹) found the Wassermann reaction to be positive in 90 per cent. of 4,000 cases of yaws with manifest lesions. Goodpasture and De Leon (1923), on testing twelve yaws patients through several months, after treatment with two injections of neosalvarsan, found that the strength of the Wassermann reaction gradually weakened until, six months after treatment, it was negative in seven cases and much weaker in the other five. The twelve patients were in the early eruptive stage of the disease when treatment was begun. Schöbl (1928¹) stated, from the results of his experimental work on Philippine monkeys, that the Wassermann reaction is indefinite and ephemeral in local yaws and that its strength and persistence depend on the duration of infection, on the number and intensity of the yaws lesions, and, to some extent, on the number of superinfections.

Administration of alcohol, either orally or by intravenous or intramuscular injection, was found by Tanabe (1928) to cause a reduction in the strength of the Wassermann reaction in yaws-infected Philippine monkeys. The action was noticeable one hour after the alcohol was given and reached its maximum in 24 to 48 hours, while in 96 hours the strength of the reaction had returned to its original intensity.

Schöbl and Tanabe (1929) found that they could produce a positive Wassermann reaction in monkeys by inoculating them with yaws material containing spirochætes, even when the material had been heated for 1 hour at 60°, or at 80°, or at 100° C. Miyao (1929¹) considered that the presence of spirochætes in the inoculum was necessary to produce a positive Wassermann reaction in experimental animals. He injected subcutaneously into monkeys filtered yaws material, into another series of monkeys skin of normal monkeys, scraped off, ground in saline and heated at 80° C. for 1 hour, and into a third series an emulsion of normal areas of skin taken from yaws-infected monkeys; in no case did a positive Wassermann reaction appear. Miyao (1929²), using heated yaws tissue, inoculated monkeys, two subcutaneously, two intraperitoneally and two intramuscularly, and found that, in the two subcutaneously inoculated monkeys some degree of reaction to the Wassermann test appeared, while there was none in the monkeys injected intraperitoneally or intramuscularly. From this experiment Miyao concluded that the skin tissue proper, and not the lymphatic or muscular tissues, is responsible for the production of the 'Wassermann reagin', but his experiment does not appear to be sufficiently extensive or conclusive to warrant this conclusion.

Flocculation Tests.

Investigations by numerous workers show, generally, that the flocculation reactions in yaws are similar to those in syphilis.

Cutaneous Test.

The luetin test was found to give positive results in yaws cases by Baermann and Heinemann (1913). They tested the action of 'Frambötin', a spirochæte-containing material from unbroken yaws papules, and found the results to be comparable with those of luetin. Schöbl (1928²) noted that, on the intradermal inoculation of infectious yaws material into immune monkeys, a 'luetin-like reaction' was obtained; this agrees with the results of the frambötin injections of Baermann and Heinemann. Kindelberger, cited by Baermann (1927²), found the luetin reaction positive in 253 out of 369 cases of gangosa. The above findings in the serum and cutaneous tests show that these tests are of no assistance in the differentiation of yaws from syphilis.

Immunization.

Very little work that is conclusive was done until, about ten years ago, American and other workers in the Philippine Islands entered on an intensive study of yaws and carried out a large amount of clinical and laboratory work, which has been recorded mostly in the *Philippine Journal of Science*. Schöbl (1928¹), from his experimental work on Philippine monkeys and from a consideration of yaws in man, finds no evidence of humoral immunity. Against the idea of humoral immunity he states that it is a common observation that monkeys become resistant to superinfection long before existing lesions heal, and these lesions are

not secluded from the general circulation of blood and lymph. In six instances he failed to influence yaws lesions in monkeys by injecting into them serum from monkeys that had gone through generalized yaws and were in a resistant condition. Alston (1911), however, claimed that he found temporary improvement in yaws in man by injecting blister fluid from other human yaws patients, who had been treated with salvarsan, but Sellards and Goodpasture (1923) were unable to confirm Alston's findings and Schöbl notes that their negative results agree with his observations in monkeys.

Schöbl's observations indicate that a resistance to reinfection or to superinfection develops slowly and appears to vary with the extent of the disease, appearing more rapidly in cases with generalized lesions than in those in which only a local yaw follows inoculation. Thus, in Philippine monkeys, he found that animals were susceptible to superinfection up to six or seven months after inoculations which produced only a single yaw, but in those which developed generalized lesions resistance appeared when cropping out of these lesions ceased. In order to discover the time at which resistance to superinfection appears, Schöbl treated with arsenical preparations monkeys at various stages of the disease and, later, re-inoculated them. Resistance began to appear at the end of the first two months of the disease and was marked about the fifth month, by which time the yaws lesions had reached the deeper layers of the skin. Schöbl found active lesions lasting for months after resistance to superinfection had become established. He concludes that it is the development of immunity that prevents the spread of yaws to other parts of the body. He considers that reinoculability cannot be used as a criterion for complete cure or sterilization of the body organism of man or animals. First of all, man, like animals, can be superinfected while infection exists in the form of local yaws, up to six months, or, where there are generalized yaws, superinfection is possible as long as metastatic dissemination continues, while, in late ulcerative forms, superinfection may be possible as long as ulcerative lesions remain active. On the other hand, while superinfection might not be possible after six months, Schöbl found that evident lesions could persist for months and that spirochaetes could be found in them long after there was complete resistance to artificial superinfection or to spontaneous generalization.

Schöbl considers that, in most of his experimental animals, the spirochaetes were arrested at the first lymphatic glands and perished there, thus being prevented from reaching the skin, the only tissue in which they produce characteristic lesions and where they can persist for long, especially in the so-called tertiary lesions. He also considers that auto-immunization is responsible for eventual localization of the disease in the skin, and for the fact that, once an ulcerative lesion has developed on the skin, no generalization takes place, although the normal skin of the animal is still susceptible to development of atypical yaws lesions. Schöbl, in the light of his recent findings in experimentally infected

monkeys, re-examined the results of experiments made on man by Sellards and Goodpasture (1923) and by Lacy and Sellards (1926). In these experiments four patients in the secondary stage of yaws were treated with neosalvarsan and six months later were inoculated with yaws material. Two developed no recognizable yaws lesions, another a localized yaw at the seat of inoculation, and the fourth a localized yaw followed by generalized yaws. Two years later the four men were reinoculated with yaws; three developed no lesion, while the fourth, who previously had produced generalized lesions, again showed localized and generalized lesions. Schöbl notes that, though at the time of the first experimental inoculation all four patients showed secondary lesions, the age of these lesions varied, being 12 and 24 months in the two patients who did not develop lesions, 8 months in the patient who developed only a localized lesion, and only 4 months in the patient who developed localized and generalized lesions. The first two men were obviously in a state of immunity, the third was partially immune and the fourth was non-immune. Schöbl concludes that a person who has had yaws for from 8 to 12 months and has had typical crops of metastatic yaws will no longer contract the disease, either naturally or experimentally, but that an individual who has had yaws for less than six months is still susceptible to superinfection. He states that it is not known how long immunity lasts, but Lacy and Sellards (1926), in their experimental work on yaws patients, showed that immunity lasts over two years, and Schöbl suggests that it may be lifelong from the fact that, in a yaws-infected area, the patients suffering from secondary yaws are usually children. Also, adult natives who have suffered from yaws consider themselves unlikely to contract the disease again, as was noted by Sellards and Goodpasture (1923), while they were investigating yaws in the Philippine Islands. In explaining the occurrence of late yaws manifestations in man, Schöbl, from the results of his experiments on monkeys, is of opinion that they may be due to a low-grade infection, with feeble reaction of the patient. In support he quotes the clinical observation of Schnitter on four ulcerative cases in the Philippine Islands, in each of which there had been an original papule with no secondaries. Also, in animals with late yaws Schöbl found that superinfection was possible and Sellards and Goodpasture found the same in man.

The Immunological Relationship of Yaws and Syphilis.

The immunological relationship of yaws and syphilis has been investigated by many workers, on man and on animals, with apparently varying results. Charlouis (1881) succeeded in inoculating syphilis on a man who was covered with a secondary yaws eruption and who stated that he had contracted the disease four years previously; a hard chancre appeared at the site of the syphilis inoculation, and, in two and a half months, a generalized syphilitic eruption. There also came under Charlouis's observation a man who was covered with a secondary yaws eruption which had

existed 'for a long time', and who had contracted a syphilitic chancre on the penis; nine weeks later, while under observation, a generalized papulo-macular eruption developed and also mucous patches in the mouth and anus. Powell (1923) described two cases of yaws which developed syphilis while under his observation in Assam. In one case the chancre appeared four months after the occurrence of the primary yaws lesion, and a secondary syphilitic eruption, which developed four months after the appearance of the chancre, lasted only a few weeks, while the generalized yaws eruption lasted about fifteen months longer. The other patient, husband of the previous one, developed a yaw, and, eight months later, he contracted a syphilitic chancre, followed four months later by a secondary syphilitic eruption; both generalized eruptions persisted till the patient's death from dysentery, about six months later.

Powell's cases come within the time when susceptibility to reinfection with yaws might still be possible, while the description of Charlouis's cases is not sufficient to show that they were not of an ulcerative type and not actively immune even to yaws.

Powell also described four cases of syphilis, one congenital, which developed yaws, and it is curious to note that, in all four, the primary lesion appeared on a broken-down gumma. Manson-Bahr (1928) suggested that saturation of a community with yaws produces a relative immunity against syphilis, and to such immunity he attributes the absence of syphilis in the natives of Fiji and neighbouring islands, where yaws is rife and where syphilis is seen in Indian immigrants. Jahnelt and Lange (1927) inoculated into a case of general paralysis of the insane yaws material from rabbits infected with a Sumatra strain of *S. pertenuis*. In fourteen days a crusted lesion appeared, in which, later, spirochaetes were found. Previously (1925) the same authors had reported failures in similar cases, in which they used two strains of *S. pertenuis* obtained from Nichols and from Brown and Pearce in America.

Schöbl and Miyao (1929) investigated the immunological relationship of yaws and syphilis in monkeys. They first examined the work of previous observers—Neisser, Baermann and Halberstadter, Halberstadter, Castellani, and Levaditi and Nattan-Larier. In this work, performed between 1906 and 1908, four monkeys, primarily inoculated with yaws, were subsequently infected with syphilis, while of eight monkeys, infected with syphilis, three were successfully inoculated with yaws. Schöbl and Miyao point out that, in these experiments, there was only one animal in which the time between the inoculations of the two diseases was more than 210 days, so that immunity to the original disease might not have developed. They also consider that there is little or no evidence that the viability of the material employed was tested on control animals, or that immunity to the primary disease was tested. Schöbl and Miyao then performed a series of experiments on the Philippine monkey, *Cynomolgus philippinensis*, having first proved this animal susceptible to the Nichols strain of *S. pallida* employed. Syphilitic material obtained from infected

rabbits was inoculated intradermally into thirteen monkeys which had recovered from yaws and had been proved immune to reinfection with yaws, while five normal monkeys were similarly inoculated with the same material to act as controls. The five normal monkeys developed syphilis, but the yaws-immune animals showed only transient lesions, resembling a positive luetin reaction. None of these luetin-like lesions showed spirochætes, but neither did some of the lesions in the normal monkeys, so Schöbl and Miyao removed lymphatic glands, in the region of the site of inoculation, and inoculated them into rabbits' testicles. Glands from the control animals infected the rabbits, but those from the yaws-immune monkeys produced no infection.

It is evident that much work in man and animals has yet to be done before the immunological relationship of yaws and syphilis can be decided.

Chemotherapy.

Remedies employed in the treatment of syphilis are used in the treatment of yaws and appear to be more active in the latter disease. Salvarsan and its derivatives give excellent results, bismuth preparations are satisfactory and are now more extensively used on account of their relative cheapness.

Antibacterial Measures.

The methods of combating yaws are treatment of cases, on as large a scale as is possible, and improvement in the living conditions of the populations, usually rural, in which the disease is prevalent. Segregation of patients, though desirable, is in most cases impracticable on the large scale in which it would have to be undertaken, sufficient hospital accommodation and funds not being at present available.

REFERENCES.

- ALSTON, H., 1911, *Brit. Med. J.*, i, 360 and 618.
 BAERMANN, G., 1927¹, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, Jena, **7**, 356; 1927², *ibid.*, p. 359.
 BAERMANN, G. & HEINEMANN, H., 1913, *Münch. med. Wschr.*, **60**, 1537.
 BALFOUR, A., 1928, *Trans. Soc. Trop. Med. Hyg.*, Lond., **22**, 295.
 CASTELLANI, A., 1905¹, *J. Ceylon. Br. Brit. Med. Ass.*, **2**, 54; 1905², *Brit. Med. J.*, ii, 1280; 1905³, *ibid.*, ii, 1430; 1907¹, *Lancet*, Lond., ii, 706; 1907², *J. Hyg.*, Camb., **7**, 558.
 CASTELLANI, A. & CHALMERS, A. J., 1919¹, *Manual of Tropical Medicine*, London (Baillière, Tindall & Cox), p. 458; 1919², *ibid.*, p. 1542.
 CHARLOUIS, M., 1881, *Vjschr. Derm. Syph.*, **8**, 431.
 FORD, W. W., 1927, *Text Book of Bacteriology*, Philadelphia and London (W. B. Saunders Coy.), p. 973.
 GOODPASTURE, E. W., 1923, *Philipp. J. Sci.*, **22**, 263.
 GOODPASTURE, E. W. & DE LEON, W., 1923, *Philipp. J. Sci.*, **22**, 221.
 HANSCHALL, H. M., 1928, *Brit. J. Venereal Dis.*, **4**, 66.
 JAHNEL, F. & LANGE, J., 1925, *Münch. med. Wschr.*, **72**, 1452; 1927, *ibid.*, **74**, 1487.
 LACY, G. R. & SELLARDS, A. W., 1926, *Philipp. J. Sci.*, **30**, 453.
 LOPEZ-RIZAL, L. & SELLARDS, A. W., 1926, *Philipp. J. Sci.*, **30**, 497.
 MACLEOD, J. M. H., 1901, *Brit. Med. J.*, ii, 797.

- MANSON-BAHR, P. H., 1928, *Brit. J. Venereal Dis.*, **4**, 44 ; 1929, *Manson's Tropical Diseases*, London (Cassell & Co.), p. 496.
- MIYAO, I., 1929¹, *Philipp. J. Sci.*, **40**, 71 ; 1929², *ibid.*, **40**, 75.
- MODDER, E. E., 1907, *J. Trop. Med.*, **10**, 187 and 361.
- MOSS, W. & BIGELOW, G., 1922, *Johns Hopk. Hosp. Bull.*, **33**, 43.
- NEISSER, A., BAERMANN, G. & HALBERSTADTER, L., 1906, *Münch. med. Wschr.*, **53**, 1337.
- NICHOLS, H. J., 1910, *J. Exp. Med.*, **12**, 616 ; 1925, *Amer. J. Trop. Med.*, **5**, 429.
- NOGUCHI, H., 1911, *Münch. med. Wschr.*, **58**, 1550 ; 1917, *Amer. J. Syph.*, **1**, 261.
- PAULET, P., 1848, *Arch. gén. Méd.*, **17**, 385.
- PEARCE, L. & BROWN, W. H., 1925, *J. Exp. Med.*, **41**, 673.
- POWELL, A., 1923, *Proc. R. Soc. Med.*, **16** (Sect. Trop. Dis.), 15.
- RAMSAY, G. C., 1925, *J. Trop. Med.*, **28**, 85.
- ROSSITER, P. S., 1912, *U.S. Nav. Med. Bull.*, **6**, 78.
- SCHLOSSBERGER, H., 1927, *Zbl. Bakt.*, Abt. I, Orig., **104**, 237*.
- SCHNITTER, F., 1921, *J. Trop. Med.*, **24**, 229.
- SCHÖBL, O., 1928¹, *Philipp. J. Sci.*, **35**, 209 ; 1928², *ibid.*, **35**, 260.
- SCHÖBL, O. & MIYAO, I., 1929, *Philipp. J. Sci.*, **40**, 91.
- SCHÖBL, O. & TANABE, B., 1929, *Philipp. J. Sci.*, **40**, 57.
- SCHÜFFNER, W., 1907, *Münch. med. Wschr.*, **54**, 1364.
- SELLARDS, A. W. & GOODPASTURE, E. W., 1923, *Philipp. J. Sci.*, **22**, 233.
- SELLARDS, A. W., LACY, G. R. & SCHÖBL, O., 1926, *Philipp. J. Sci.*, **30**, 463.
- SHARPE, W. S., 1928, *Trans. Soc. Trop. Med. Hyg.*, Lond., **22**, 293.
- STITT, E. R., 1929, *Tropical Diseases*, London (H. K. Lewis & Co.), p. 156.
- TANABE, B., 1928, *Philipp. J. Sci.*, **37**, 247.
- VON DEM BORNE, E. W. K., 1907, *J. Trop. Med.*, **10**, 345.
- WENYON, C. M., 1926, *Protozoology*, London (Baillière, Tindall & Cox), **2**, 1269.
- YASUYAMA, K., 1928, *Philipp. J. Sci.*, **35**, 333.

CHAPTER XI. THE NORMAL BACTERIAL FLORA OF MAN.

BY JOHN CRUICKSHANK (UNIVERSITY OF ABERDEEN) AND
ROBERT CRUICKSHANK (THE UNIVERSITY AND
ROYAL INFIRMARY, GLASGOW).

The Bacterial Flora of the Cutaneous Surfaces.

DESPITE the exposure of the skin to contamination from bacteria in the air, dust, secretions from mouth and nose, and from the soil, cultures from the exposed parts of the body yield relatively few bacteria. These organisms are for the most part non-pathogenic, e.g. *Staphylococcus albus*, *M. tetragenus*, and diphtheroids, and include, in addition, the bacteria found in the air and dust of rooms, pigment-forming cocci, moulds and spore-bearing organisms. The acid-fast smegma bacillus has been recovered from the skin and a specific micrococcus is described from the scurf of the scalp (Gordon, 1903-4). Bacteria such as *Staphylococcus epidermidis albus* of Welch and the acne bacillus seem to be present in the deeper layers of the skin, in the ducts of sweat-glands and in the hair-follicles.

Pathogenic organisms such as *B. typhosus* and the tubercle bacillus may be isolated from the hands, and particularly from under the nails, of those in contact with acute cases of these infections. Winslow (1903-4) found coliform bacilli on the hands of 5 to 10 per cent. of the students at Boston, and interpreted his findings as an indication of fæcal contamination. On the body-surfaces covered by clothing bacteria are more abundantly present. The frequent finding of enterococci in gunshot wounds during the war indicates how widely these organisms may be disseminated on the skin. In the groins and on the rugose skin of the scrotum smegma bacilli are commonly present and organisms of intestinal origin may usually be recovered from the skin of the buttocks. On the skin of the feet particularly, where there is lack of attention to general hygiene, numerous staphylococci and streptococci may be found; even tetanus spores have been recovered. In the external auditory meatus *Aspergillus niger* and *Aspergillus fumigatus* may cause a particular type of lesion and staphylococcal lesions are common.

Some interesting findings of Smith (1928) confirming the work of Michael (1923) are worthy of record in connection with pyogenic infections of the skin. Where pyoderma was present, cultures from the fingers usually yielded hæmolytic staphylococci, but similar cultures were negative in the great majority of a large number of controls not suffering from pyogenic skin lesions. These findings do not support the view that

pyogenic cocci are frequently found on the normal skin, and suggest that the pyodermias are definitely infectious from one person to another.

External genitalia of male. Smears made from the preputial secretions or from the surface of the glans penis in individuals who observe ordinary cleanliness reveal numerous smegma bacilli, diphtheroids, and Gram-positive and Gram-negative cocci. Where the secretion is allowed to collect, fusiform bacilli, spirilla and spirochætes may be abundantly present. The fusiform bacilli are similar to those found as saprophytes in the mouth. Noguchi (1918) made a special study of the spirochætal flora of the normal male genitalia and described three varieties of spirochætes isolated from the preputial secretions or the glans, (a) *Spironema refringens*, a large coarse type which is not always present, (b) a medium-sized spirochæte identical with *Treponema calligyrum*, which he first isolated from a condyloma and which closely resembles *T. pallidum*, and (c) *Treponema minutum* (subsequently named *T. genitalis*), a hitherto undescribed variety, very like *T. microdentium* but differing from it in certain cultural characters and also serologically. Stoddard (1917) had previously reported the occurrence of spirochætes in the urine and in the urethral orifice of healthy individuals; some of these were morphologically indistinguishable from the leptospiræ. Noguchi did not find leptospira-like organisms either in the urine or in the urethra. In view of Stoddard's work the presence of spirochætes in the urine cannot be taken as conclusive evidence of a spirochætal infection without detailed examination of the organism.

External genitalia of female. The bacteria of the labia majora and minora are related to the bacterial flora of the immediate neighbourhood, which is naturally dominated by organisms of intestinal origin. The numbers and variety of bacteria are largely dependent on the observance or otherwise of cleanliness of the parts; *Staphylococcus albus*, diphtheroids, coliform organisms, *Enterococcus* and *Streptococcus viridans*, *Micrococcus tetragenus* and yeasts have all been isolated. In the vulva a modified bacterial life exists and organisms are less numerous. The accumulation of the mucoid vaginal secretions favours the growth of the smegma bacillus. Fusiform bacilli and spirochætes are frequently present about the clitoris (Pilot and Kanter, 1923), and may play a secondary part in inflammatory conditions and ulcerations about the vulva. According to Noguchi and Kaliski (1918) the spirochætes of the normal female genitalia are similar to those found in the male.

REFERENCES.

- GORDON, M. H., 1903-4, *Rep. Med. Off. Loc. Govt. Bd.*, 388.
MICHAEL, M., 1923, *Derm. Z.*, **39**, 329.
NOGUCHI, H., 1918, *J. Exp. Med.*, **27**, 667.
NOGUCHI, H. & KALISKI, D. J., 1918, *J. Exp. Med.*, **28**, 559.
PILOT, I. & KANTER, A. E., 1923, *J. Infect. Dis.*, **32**, 204.
SMITH, J. F., 1928, *Brit. J. Derm.*, **40**, 483.
STODDARD, J. L., 1917, *Brit. Med. J.*, ii, 416.
WINSLOW, C. E. A., 1903-4, *J. Med. Res.*, **10**, 463.

Bacterial Flora of the Normal Mouth.

The passage of inspired air, ingestion of food-stuffs, and contact with the exposed skin of the face in the neighbourhood of the lips, ensure the entry into the mouth of many different organisms. Inside the mouth the presence of abundant moisture and a temperature akin to that of the body afford very favourable conditions for the growth of many organisms. It is, therefore, not surprising that the list of bacteria which have been found in this situation is a most extensive one.

At birth the face and lips of the child frequently become contaminated with organisms from the vagina and perineal region of the mother. Schweitzer (1919), for example, records the finding of Döderlein's vaginal bacillus in the mouths of 46 per cent. of newly born children, whereas in children delivered by Cæsarean section, he found no evidence of vaginal organisms. Others have found that immediately after birth, the mouth of the child is sterile (Bonnaire and Keim, 1900; Podbielskij, 1890; Jeannin, 1904), but that within 6 to 10 hours staphylococci, *B. coli*, and *Staphylococcus parvulus* are present and somewhat later enterococci, *Streptococcus salivarius*, *Micrococcus candidans* and various sarcinæ appear. A great increase in numbers occurs during the second day, partly as a result of the inception of feeding. By cultural methods at this time *B. coli*, *B. lactis aerogenes*, *Staphylococcus albus* and small cocco-bacilli have been obtained, and, in a few cases, *Pneumococcus*, *B. bifidus* and *Staphylococcus parvulus*. At a later date streptococci, staphylococci and coliform organisms predominate, and it is stated (Brailovsky-Lounkevitch, 1915) that pneumococci and pyogenic cocci are found only in the mouths of children born in the wards of maternity hospitals. Unlike the bacterial flora of the infant's intestine no dramatic change occurs in the flora of the infant's mouth as a result of feeding. The organisms in the mouth are derived from the air (sarcinæ, pigmented cocci, *B. subtilis*, *B. mesentericus*, &c.), and from the mother (*Streptococcus salivarius*, *Staphylococcus albus*, *B. lactis aerogenes*, *B. acidophilus*). Strict anaerobes are very rarely found. After the twelfth day from birth *Streptococcus salivarius* is found as the single constant species, and there is marked diminution in the numbers of the organisms which are derived from the air. *Pneumococcus* and *Streptococcus pyogenes* may be encountered in a small proportion of cases.

Whereas in the suckling the tongue, cheeks and gums present the same bacteriological picture, this is not the case after the onset of dentition. The teeth provide roughnesses and cavities for the deposition and retention of particles of food, and as a result, there is a great development of anaerobes. Smears from the cheeks and palate show a bacterial flora similar to that of the suckling, and among desquamated cell plaques, many round or oval cocci and a few Gram-negative or Gram-positive bacilli are visible; the tongue, in addition, shows leptothrix and a few spirochætes and vibrios, but smears from the gums show epithelial cells, frequently degenerate in appearance, masses of cocci, many long and thick

filaments, bacilli of different kinds and fairly numerous spirochætes. Cultures from the gums have yielded *Streptococcus salivarius*, *M. candidans*, variously named streptococci, *Pneumococcus*, *B. coli* and different anaerobes (*Staphylococcus parvulus*, *B. bifidus*, *Leptothrix buccalis*, *Spirillum sputigenum*) and various vibrios. With advancing years tangled filaments of leptothrix are found on the gums at the margins of the teeth along with numerous vibrios and spirochætal forms, cocci and bacilli. Masses of leptothrix are also found in the tartar which accumulates. A great many different organisms have been found, but the more or less constant flora consists of *Streptococcus salivarius*, *Leptothrix*, spirochætes, spirilla, *Staphylococcus parvulus* and various anaerobic bacilli. *B. fusiformis* may also be found.

In the adult the picture becomes even more complicated. Miller (1892), for example, has described over a hundred different varieties of organisms. According to Braïlovsky-Lounkevitch (1915) the examination of the cheeks, tongue and palate give results suggestive of those found in the suckling, with the addition of certain organisms from the tongue and gums. Small and large Gram-positive diplococci predominate in films from these situations, together with a few leptothrix forms and spirilla. On the tongue the same diplococci are found in great numbers in addition to masses of staphylococci, short and long thin rods, spirochætes in variable numbers, leptothrix, filaments and vibrios. On the gums few cocci are found and other organisms occur in variable numbers in different individuals; in some mouths great numbers of slender Gram-negative rods predominate, in others filaments of leptothrix, in others spirochætes. Organisms are present in greater abundance among the villi on the back of the tongue than on the tip. Between the teeth anaerobes almost exclusively are found, and in different individuals there is little difference in the kinds which are encountered in films although the relative numbers may show great variation, leptothrix threads, spirochætes, fusiform bacilli, or small or large rods predominating in certain cases. The habitual flora of the adult is, in fact, much the same as that of the child after dentition, but greater variations occur according to the state of the mouth and the condition of the teeth. The fundamental flora consists of *Streptococcus salivarius*, the predominating form, together with various anaerobes, *Leptothrix*, spirilla, vibrios, *Staphylococcus parvulus*, and others.

The state of the teeth, their arrangement in the mouth, the presence of dental caries and cavities may all affect the picture in varying degrees, and a careful hygiene of the mouth with removal of all disease in the teeth effects a very considerable reduction in the number of organisms, especially of spirochætal and vibrio forms. When the teeth are completely removed, the flora of the mouth generally returns to the type of that found in the suckling infant.

Pathogenic organisms, e.g. *Pneumococcus* and *Streptococcus pyogenes*, are encountered in the normal mouth, but the finding of pneumococci depends greatly upon the technique employed, the injection of the mouth

secretions into mice giving a percentage of positive results much higher than any method of direct culture, and thus the positive results of different workers vary from 5 to 100 per cent. of the cases examined.

In cultures many of the mouth organisms are active in fermenting a variety of substances (starch, sugar, protein) and it has been suggested that their presence is beneficial to the individual, and that putrefactive and pathogenic organisms are inhibited in development. On the other hand, the localized production of acids in contact with the enamel of the teeth has been held to be a factor in the causation of dental caries. *B. acidophilus odontolyticus* (McIntosh, James and Barlow, 1925) can be found in the mouth when carious teeth are present, but its causal relationship to caries is extremely difficult to establish.

The experimental observations of Bloomfield (1919, 1920, 1921), who inoculated the throat and mouth with various bacteria, suggest that foreign organisms (*B. coli*, staphylococci, sarcinæ, *B. influenzae*) are rapidly disposed of by the flushing mechanism of the mouth secretions. He expresses the opinion that, except for the normal flora, bacteria do not as a rule grow freely on the mucous membranes of the mouth and throat, and that special conditions are necessary to account for the presence of foreign organisms, either a local infection or a transient invasion. He found that the most common organisms on the tongue were Gram-negative cocci, that non-hæmolytic streptococci were constantly present, and, less frequently, diphtheroids. Hæmolytic influenza bacilli, hæmolytic streptococci and *Staphylococcus albus* were present only as transients. The same flora was found on the tonsils and in the pharynx, but the transients were much more numerous in these situations. He found that repeated cultures from the same individual were required to establish the normal flora.

Naeslund (1925) has isolated a variety of *Actinomyces* from the mouth, using as a medium a mixture of sterilized saliva and agar, but was unable to distinguish clearly the various types. He believes that the anaerobic forms are part of the normal flora and the aerobic strains only transient invaders.

The organism described by Lewkowicz (1901) which he obtained from the mouth of a healthy infant and to which he gave the name *M. gazogenes alkalescens anaerobius* appears to be related to *Staphylococcus parvulus* described by Veillon and Zuber in 1898 and by Rist (1901) in cases of gangrenous appendicitis. Holman and Krock (1923) describe a small coccus of similar characters which is frequently present in the mouth of the human subject and of rabbits and guinea-pigs, the cultures of which were found to be filterable through Mandler and certain Berkefeld filters. They suggested that the organism was related to *B. pneumosintes* (Olitsky and Gates, 1921). Hall and Howitt (1925) have isolated 24 strains of similar organisms from 35 samples of human saliva, but disagree with Holman and Krock as to the filterability and nomenclature of the organisms. The relationships of these organisms to *Staphylococcus parvulus* and other

small anaerobic cocci obtained from other sites in the body are discussed by Branham (1928).

A number of organisms found only in the mouth have been described by Goadby and other workers, but there is considerable uncertainty regarding the characters of many of them. Several varieties of large cocci, some in chains which stain a deep red with iodine, can be found in films from the mouth and have been variously named (*Iodococcus parvus*, *Iodococcus magnus*, *Iodococcus vaginatus*), but their relationships are not clearly established. Large bacilli which react similarly with iodine are also present and are generally grouped together as *B. buccalis maximus*. Strains of leptothrix are described (Miller, Goadby, Brailovsky-Lounkevitch), but further work is necessary to establish all their characters and relationships. Pigment-producing cocci and bacilli are also frequently encountered presumably derived from the inspired air.

The presence of sporulating anaerobes in the saliva has been examined by Hall (1925). *B. welchii*, *B. tetani*, *B. bifementans* and *B. tetanomorphus* have been recovered but are found only as occasional transients.

The bacterial flora of the mouth is obviously extremely complicated and in addition may undergo variation from time to time, and these factors at present offer almost insuperable difficulties in the investigation of pathological lesions of the gums and teeth. The view which is widely held by clinicians that many general bacterial infections and toxæmias in the human subject have their portal of entry in carious or dead teeth and are disseminated by the associated lymphatics rests upon slender bacteriological foundations, and the factors just mentioned render research on this aspect of medicine a subject of great complexity.

B. FUSIFORMIS.

The association of fusiform bacilli and spirilla with certain ulcero-membranous gangrenous conditions in the mouth and elsewhere in the body is well known. *B. fusiformis*, however, is present so frequently as an apparently harmless saprophyte in the mouth, about the external genitalia, &c., that a description of its characters seems desirable in this section on the normal flora.

B. fusiformis is classified by Bergey (1926) in Group III of the Actinomycetales. The fusiform bacilli are most closely related morphologically to the corynebacteria and Libman has suggested for them the name *Fusobacterium*: Knorr (1922), in the study of a large number of strains, divided the fusiform bacilli into three groups according to their morphological and cultural characters—*Fusobacterium plaut-vincenti*, *Fusobacterium nucleatum* and *Fusobacterium polymorphum*.

The first description of fusiform bacilli was by Babes (1884), who found them in a diphtheria-like infection of the fauces. Plaut (1894) reported the occurrence of fusiform bacilli in ulcero-membranous lesions of the mouth. Two years later, Vincent described fusiform bacilli and spirilla in hospital gangrene infections and in 1898 gave a clinical description of

14 cases of ulcero-membranous angina in all of which fusiform bacilli and spirilla forms were demonstrable. The lesion was subsequently called Vincent's angina, and the fusiform bacillus, named Vincent's or Plaut-Vincent's bacillus, was believed to be causally related. Fusiform bacilli have since been found in a great variety of ulcerative and gangrenous conditions—for example, in ulcerative stomatitis, in gingivitis (McKinstry, 1918), in cancrum oris and noma, in erosive balanitis and chancres, in abscesses of lung, brain and appendix, from the blood in certain conditions and in chronic foetid otitis media (Pilot and Pearlman, 1923). In addition, *B. fusiformis* is frequently present about the teeth and gums intimately involved in the tartar, in the crypts of tonsils (Pilot and Brams, 1923) forming actinomycotic-like granules (pharyngo-mycosis), and on the external genitalia (particularly in preputial secretions and about the clitoris) of healthy individuals. The organism seems to thrive best where there is decaying organic matter and some degree of anaerobiosis.

Morphology. Although there are considerable variations in the morphological characters of *B. fusiformis*, it may be described as a Gram-negative bacillus 6 to 12 μ long and 0.6 to 0.9 μ wide, thickened in the middle and pointed at one or both ends, straight or slightly curved, occurring singly or in diplobacillary form. It is non-motile and non-sporogenous. Stained by methylene blue the organism may show granules and vacuoles, accentuating its resemblance to the corynebacteria: with Giemsa's stain the bacillus appears light bluish-red and in the centre a nuclear-like body—believed by some to be a true nucleus (Hoelling, 1910; Mühlens and Hartmann, 1906)—is often demonstrable. Varney (1927) recognized among his strains four different morphological types, (a) a narrow filamentous bacillus, (b) a short slender form, (c) a broad stubby bacillus, and (d) one forming spiral elements due to chain-formation.

Films from culture may also show considerable variation in the length and thickness of a supposedly pure strain of the organism; short chains and filaments are common in young cultures, while deep colonies of older cultures in serum-agar may yield many long spiral filaments, up to 200 μ in length, unbranched and forming tangled thread-like masses. When grown on dissimilar lots of media, a particular strain may undergo great changes in its morphological characters.

Cultural characters. *B. fusiformis* requires strict anaerobiosis for its growth in pure culture, especially where surface growth is desired; in symbiosis with other organisms, however, in particular with streptococci, the organism may multiply in ordinary nutrient broth under semi-anaerobic conditions. In pure culture, *B. fusiformis* is serophilic, requiring blood-serum, or ascitic or other body-fluid for its growth and maintenance: some strains grow in subculture on serum-free media enriched with sugar, but for continued vigorous development and security of transfer, a serum medium is essential. A neutral or slightly alkaline reaction gives the optimum conditions for growth. In the depth of serum-agar growth appears

in 48 hours as very small ($\frac{1}{2}$ to 1 mm. in size) round or oval opaque colonies with smooth edges and fine prolongations into the surrounding medium; or the colony may have a dark centre with a lighter yellowish granular periphery. There may be a crop of smaller colonies surrounding a large one. Surface colonies are dew-drop-like raised, greyish (or greyish-yellow) in colour, with a felted surface, and may show one or two concentric rings. In fluid media growth is meagre and settles as a whitish flocculent sediment. After frequent subculture the organism may show considerable viability. It has been recovered in culture after 3 to 4 months in the ice-chest, and, according to Knorr, remains viable for 3 months under ordinary cultural conditions.

Biochemical reactions. Despite the putrid odour usually associated with cultures of *B. fusiformis*, the organism in the pure state seems not to be proteolytic and has no disagreeable odour nor does it form H_2S or indole (Varney, 1927). Krumwiede and Pratt (1913, 1914), however, state that their strains formed both H_2S and indole. Many strains have little or no saccharolytic activity and there is no formation of gas in the sugars that are fermented. A division of their strains into saccharose-fermenters and non-saccharose-fermenters was made by Krumwiede and Pratt. *B. fusiformis* has no reducing powers and is non-hæmolytic.

Serological classification. Varney examined 18 strains of *B. fusiformis* isolated from both healthy and diseased tissues of the mouth. In an attempt to classify his strains serologically, he prepared antisera to four types which differed from one another in their morphological and cultural characters. He was able by serological reactions to divide his strains into three groups, but only the members of the first two were specifically agglutinated by antisera prepared against representative strains: Type III was not agglutinated by any of the antisera, while a fourth group was also non-agglutinable and differed widely in its morphology from the preceding types.

The transformation of B. fusiformis into spirilla. The association of fusiform bacilli and spirilla is a common one and is usually regarded as being a symbiotic relationship. The suggestion that the spirillar forms are derived from, or form a stage in the evolution of, *B. fusiformis* was first made by Tunncliffe (1906), who observed non-motile spirilla appearing in apparently pure cultures—young as well as old—of *B. fusiformis*. A similar phenomenon was described by the same worker in 1911, and her observations received confirmation from Larson and Barron (1913). In 1923, Tunncliffe described in detail the characters of a pleomorphic fusiform bacillus isolated from a healthy tonsil. Filamentous forms appeared in 24 to 48 hours and true branching was observed on two occasions: at the end of 1 to 2 weeks motile spirilla were seen, particularly in cultures from blood-agar medium or acidified ascitic-broth. The spirilla were formed inside the fusiform bacilli and escaped either at an extremity or by lysis of the parent organism. Conversely, spirilla could become transformed into fusiform bacilli.

Tunnickliff's findings have not been generally accepted (Pratt, 1927; Semple *et al.*, 1919), and in view of the difficulty of obtaining pure cultures of *B. fusiformis* a possible explanation of her observations is that her original culture was a mixture of fusiform bacilli and spirilla. Some support to Tunnickliff's thesis has, however, been given by Sanarelli (1927), who from his observations concluded that the fuso-bacteria are derived from the spirochætes, and have become immobile under the influence of factors such as the metabolic products of associated bacteria or the prolonged contact with atmospheric oxygen. The bulk of opinion still inclines to the belief in a symbiotic relationship between *B. fusiformis* and the spirilla, rather than to an evolutionary process of one organism into the other.

Pathogenicity. The association of fusiform bacilli with a wide variety of inflammatory, ulcerative, and gangrenous conditions, and its occasional presence in pure culture in brain abscesses, in pulmonary lesions, or in the blood, has been regarded as sufficient evidence of its pathogenicity. Some scepticism is permissible with regard to it as the primary causative factor in such lesions, since the organism is a common saprophyte in certain parts of the body in health (mouth, tonsils, external genitalia). It is more probable that *B. fusiformis* is a secondary invader of tissues already diseased or devitalized. There seems little doubt that the organism helps to maintain or further the progress of ulcerative and gangrenous conditions.

REFERENCES.

- BABES, 1884, cited from 1913, *Handb. d. path. Mikroorg.* hrsg. Kolle u. Wassermann, **5**, 1003.
 BERGEY, D. H., 1926, *Manual of Determinative Bacteriology*, London.
 BLOOMFIELD, A. L., 1919, *Johns Hopk. Hosp. Bull.*, **30**, 317; 1920, *ibid.*, **31**, 14, 85 and 203; 1921, *ibid.*, **32**, 10, 121 and 290.
 BONNAIRE, E. & KEIM, G., 1900, *Pr. méd.*, **8**, 61.
 BRAILOVSKY-LOUNKEVITCH, Z. A., 1915, *Ann. Inst. Pasteur*, **29**, 379.
 BRANHAM, S. E., 1928, *J. Infect. Dis.*, **42**, 230.
 GOADBY, K. W., 1903, *The Mycology of the Mouth*, London.
 HALL, I. C., 1925, *J. Infect. Dis.*, **37**, 87.
 HALL, I. C. & HOWITT, B., 1925, *J. Infect. Dis.*, **37**, 112.
 HOELLING, A., 1910, *Arch. Protistenk.*, **19**, 239.
 HOLMAN, W. L. & KROCK, F. H., 1923, *Amer. J. Hyg.*, **3**, 487.
 JEANNIN, C., 1904, *Obstétrique*, **9**, 322.
 KNORR, M., 1922, *Zbl. Bakt.*, Abt. I, Orig., **89**, 4.
 KRUMWIEDE, C. JR. & PRATT, J. S., 1913, *J. Infect. Dis.*, **12**, 199; 1914, *ibid.*, **13**, 438.
 LARSON, W. P. & BARRON, M., 1913, *J. Infect. Dis.*, **13**, 429.
 LEWKOWICZ, X., 1901, *Arch. méd. exp.*, **13**, 633.
 MCINTOSH, J., JAMES, W. W. & LAZARUS-BARLOW, P., 1925, *Brit. J. Exp. Path.*, **6**, 260.
 MCKINSTRY, W. H., 1918, *R.A.M.C. Jl.*, **30**, 274.
 MILLER, W. B., 1892, *Die Mikroorganismen der Mundhöhle*, Leipzig.
 MÜHLENS, P., 1905, *Deuts. med. Wschr.*, **38**, 136.
 MÜHLENS, P. & HARTMANN, M., 1906, *Z. Hyg. InfektKr.*, **55**, 81.
 NAESLUND, C., 1925, *Acta path. Microbiol. Scand.*, **2**, 110.
 OLITSKY, P. K. & GATES, F. L., 1921, *J. Exp. Med.*, **33**, 713; 1922, *ibid.*, **35**, 813.
 PILOT, I. & BRAMS, I., 1923, *J. Infect. Dis.*, **33**, 134.

- PILOT, I. & PEARLMAN, S. J., 1923, *J. Infect. Dis.*, **33**, 139.
 PLAUT, H. C., 1894, *Deuts. med. Wschr.*, **20**, 920.
 PODBIELSKI, A., 1890, cited from 1891, *Zbl. Bakt.*, **9**, 617.
 PRATT, J. S., 1927, *J. Infect. Dis.*, **41**, 461.
 RIST, E., 1901, *Zbl. Bakt.*, Abt. I, Orig., **30**, 287.
 SANARELLI, G., 1927, *C.R. Soc. Biol.*, Paris, **96**, 1136.
 SCHWEITZER, B., 1919, *Zbl. Gynäk.*, **43**, 641.
 SEMPLE, D., PRICE-JONES, C., DIGBY, L., 1919, *R.A.M.C. Jl.*, **33**, 281.
 TUNNICLIFF, R., 1906, *J. Infect. Dis.*, **3**, 148; 1911, *ibid.*, **8**, 316; 1923, *ibid.*, **33**, 147.
 VARNEY, P. L., 1927, *J. Bact.*, **13**, 275.
 VEILLON & ZUBER, 1898, *Arch. méd. exp.*, **10**, 517.
 VINCENT, H., 1896, *Ann. Inst. Pasteur*, **10**, 488; 1898, *Sem. méd.*, **18**, 109.

The Bacterial Flora of the Upper Respiratory Tract.

In the discussion of the bacterial flora of the normal healthy air-passages separate consideration will be given to (a) the vestibule and nasal fossæ, (b) the posterior nares and nasopharynx, and (c) the trachea and bronchi. The vestibule is that region at the entrance to the nose which is lined by the vibrissæ and squamous stratified epithelium, and corresponds to the anterior nares proper. The nasal fossæ, with a mucosal lining, extend backwards to the choanal apertures and are continued posteriorly into the nasopharynx. Bacteriological examinations are concerned chiefly with the anterior—and more accessible—part of the nasal cavities, and when reference is made in the text to the nasal fossæ the anterior part of them is meant.

THE VESTIBULE AND NASAL FOSSÆ.

In 1895, St. Clair Thomson and Hewlett—whose paper in the *Medico-Chirurgical Transactions* fully reviews the earlier literature—concluded from the bacteriological examination of the noses of healthy individuals and from experiments to determine the fate of inspired organisms that 'the interior of the great majority of normal nasal cavities is perfectly aseptic', but that 'the vestibules of the nares, the vibrissæ lining them, and all crusts formed there are commonly swarming with bacteria'. These statements were directly opposed to the belief, which is still widely held, that the nasal cavities even in health are teeming with micro-organisms. Despite contradictory evidence, however, their work is substantiated by an increasing body of investigations on the flora of the nose in health.

The reports of many of the early workers in this field are directly opposed to the conception of the nasal cavities as being free from bacteria. It is probable that the examinations were not conducted in individuals with healthy nasal cavities or with the necessary precautions against contamination from the external parts. Contamination from organisms in the vestibule or by the vibrissæ lining it must be carefully avoided either by the use of a nasal speculum or by cutting off the vibrissæ and carefully disinfecting the entrance. A swab or a platinum loop may then be inserted into the nasal cavity, rubbed on the mucosa and

subsequently inoculated on suitable media. Under such conditions the anterior nares have been found to be either germ-free or practically so. Küster and Wössner (1913), for example, reported the nasal mucosa sterile in 2 of 100 cases examined, and in the remaining 98, the number of colonies was scanty, as few as four colonies being commonly present on the inoculated media. Avirulent diphtheroids were isolated from only two of their cases. In a more recent examination of the nasal flora of 50 healthy school-children (Harvey, 1928), cultures on blood-agar of a saline suspension of the nasal swabbing were negative in 10 cases, and in 24 there was scanty growth, 13 of the plates showing only 2 to 5 colonies. Among the organisms recovered, *Staphylococcus albus* was most frequently represented, *Staphylococcus aureus*, *Streptococcus viridans* and the *Pneumococcus* were rarely present, while non-hæmolytic streptococci of γ type were more commonly isolated. Neither *C. diphtheriæ* nor Hofmann's bacillus was recovered from any of the cases. Neumann (1902), on the other hand, investigated the nasal flora of 111 healthy individuals as a preliminary to a bacteriological study of acute rhinitis and isolated diphtheroids and *Staphylococcus albus* from 98 to 100 per cent. of the cases: *Staphylococcus aureus* and *citreus*, *B. coli* and moulds were recovered from a large proportion, and *Pneumococcus*, Friedländer's bacillus, and *B. ozaenæ* from a smaller number of his cases. Park and Wright (1898), in the examination of 36 persons with 'normal or nearly normal nasal mucosa', reported the isolation of numerous bacteria from the nose in all but six of their cases.

The presence of diphtheria-like bacilli in the nose has been regarded as of common occurrence and little significance: our own experience of routine nasal cultures suggests that in the absence of any inflammatory condition or structural deformity in the nose or a concurrent faucial or laryngeal diphtheria, diphtheroids and organisms morphologically identical with *C. diphtheriæ* are rarely present in the nasal fossæ, although Hofmann's bacillus is a common inhabitant of the vestibule. The general tendency for abnormal mucous membranes to favour the development of a bacterial flora which is not causally related to the pathological process is demonstrated in such conditions as ozaena and the common cold. Diphtheroids, Friedländer's bacillus, *Micrococcus catarrhalis*, staphylococci, and streptococci may all be abundantly present in the anterior part of the nasal fossæ in these affections. Similarly, the nasal carrier of diphtheria, who usually presents some pathological condition of the nose, harbours there a rich flora of heterogeneous type along with virulent *C. diphtheriæ*. The establishment of these bacteria is probably related to the absence of the sterilizing mechanism of the nasal mucosa (to be discussed later), since the restoration of a healthy mucosa in the case of the nasal diphtheria carrier eliminates not only *C. diphtheriæ* but also the concomitant flora (McCartney and Harvey, 1928).

The occasional presence in the nose of such pathogens as the meningococcus, pneumococcus, streptococcus and the tubercle bacillus has been

reported: Jones (cited by Küster), for example, demonstrated tubercle bacilli by animal inoculation in the nasal secretion of 3 out of 31 healthy persons. Meningococci, pneumococci and *C. diphtheriæ* may, of course, be recovered from the nasal fossæ of healthy contacts, particularly during epidemic periods, although the pneumococcus and the meningococcus find the nasopharynx a more congenial habitat. Similarly the virus of poliomyelitis and probably of other infections, may be carried for short periods in the noses of healthy individuals.

The Elimination of Bacteria from the Nose.

If the comparative sterility of the nasal fossæ be accepted, the mechanism of removal of invading bacteria requires explanation. It has been estimated that 4,000 to 15,000 organisms—the numbers varying with the environment—may be inspired every hour and yet experiment has shown that the tracheal air and mucus is germ-free. Several factors contribute to this process of sterilization.

The anatomic structure of the nose. The first barrier to the entrance of organisms during inspiration is the filter of moist vibrissæ lining the entrance to the anterior nares. Dust particles, bacteria, and other particulate matter are caught and deposited in the vestibule, and only forcible inspiration in an atmosphere heavily charged with bacteria results in the passage of appreciable numbers of organisms into the posterior part of the nasal fossæ. The projecting turbinates and the general anatomic structure of the nose ensure a rapid trapping on the moist nasal mucosa of any bacteria which may pass the first line of defences.

The mechanical action of the nasal secretion and the ciliated epithelium. Bacteria or other foreign particles present on the nasal mucous membrane are continually washed out mechanically by the nasal secretion, or if the mucosa be sufficiently irritated, more forcibly ejected by the process of sneezing. Mucus seems to lend its aid by enveloping the foreign particles (Yates, 1924). In addition, animal experiments have shown that the ciliated epithelium of the nares helps in the process of removal of bacteria by a mass-action which carries the foreign particle back towards the nasopharynx (Cowdry, 1928).

The auto-sterilizing power of the nasal mucous membrane Experimenters are not in agreement about the bactericidal action of the nasal secretion *in vitro*. Wurtz and Lermoyez (1893) described the nasal mucus as having distinct bactericidal powers, and Schousboe (1901) stated that the sterilized secretion was lethal to certain pathogenic organisms (*B. anthracis*, staphylococci, streptococci, &c.), but most workers deny it such powers. Thomson and Hewlett (1896), however, demonstrated a bacteriostatic action of the nasal secretion, and, similarly, Park and Wright (1898) found it to be a very poor culture medium. Recently, Fleming (1922) has shown that the nasal as well as other body secretions contain a lytic agent (lysozyme) which destroys many air organisms and particularly *M. lysodeikticus* (see Lysozyme, Vol. VI, p. 42).

The nasal mucosa itself seems to possess remarkable bactericidal properties. Thomson and Hewlett in their early work (1896) found that if a loopful of *B. prodigiosus* were placed on the nasal septum viable organisms could not be recovered from the nasal mucosa at the end of two hours. In recent experiments on guinea-pigs, rabbits, dogs and man, Arnold, Ostrom and Singer (1928), using *B. coli* or *B. prodigiosus*, have proved that 90 to 95 per cent. of the viable bacteria placed on the mucous membrane of the nose by spraying were destroyed in 5 to 10 minutes. Repeated applications of the organism did not cause any exhaustion of the mechanism. Although no theory is advanced by the authors as to the nature of the sterilizing action it seems that most of the organisms are killed within the nose since few were recovered from the nasopharynx. There is evidence that the products of dead organisms placed on the nasal mucosa may be absorbed, as demonstrated by the appearance of specific antibodies in the blood (Bull and McKee, 1928).

Phagocytosis. It is doubtful and difficult to prove whether leucocytes play any part in the removal of bacteria from the nose.

NASOPHARYNX.

Cultures from the nasopharynx are usually taken with West's tube or some modification of it to avoid contamination from the mouth and throat. Aerobic cultures from the upper part of the posterior nares are usually sterile, although the meningococcus, which may be present in 2 to 5 per cent. of the healthy populace (Zinsser, 1927), should be sought for in this region. Care must be taken to differentiate it from other Gram-negative cocci more frequently present in the nasopharynx [see recent studies by Wilson (1928), and Wilson and Smith (1928)] such as *M. catarrhalis*, *M. pharyngis siccus*, certain chromogenic cocci of the same group, and anaerobic Gram-negative cocci of indeterminate character. Pneumococci have also been recovered from the nasopharynx of a proportion of healthy people, and when typed have fallen mostly into the heterogeneous Group IV. The necessity for distinguishing pneumococcus from another normal inhabitant of the mouth and nasopharynx, *Streptococcus salivarius*, by testing the solubility of the organism in bile and its virulence for white mice, needs to be emphasized, since identification by the ordinary morphological and cultural characters is frequently not possible (Whittle, 1928). Diphtheroids and hæmophilic bacteria (Bloomfield, 1922) and various anaerobic organisms have been recovered from the healthy nasopharynx. Among the latter are a filter-passing bacterium of the type of *B. pneumosintes*, a Gram-negative hæmolytic diplococcus, and a coccus apparently identical with *Staphylococcus parvulus* (Branham, 1928), which, however, is more common in the mouth.

The importance imputed to paranasal sinus infections in the causation of morbid conditions elsewhere in the body has stimulated bacteriological examinations of these cavities during health and particularly in animals (Calamida and Bertarelli, 1902). These authors almost invariably found

that healthy sinuses were bacteriologically sterile, and even when organisms were introduced the sinuses were germ-free in 24 hours, a phenomenon probably associated with the known activity of the ciliated epithelium in rejecting or ejecting invading foreign particles (Yates, 1924).

THE TRACHEA AND BRONCHI.

The trachea, bronchi, and pulmonary tissues are sterile during health. (In 1868 Lister had remarked on the absence of 'decomposition' in injuries to the lungs from fractured ribs.) Should any organisms reach the trachea, however, they may (*a*) be mechanically removed by the ciliated epithelium which conveys foreign particles up towards the larynx, (*b*) be carried away by lymphatic drainage, (*c*) die *in situ*, or (*d*) if the organisms be virulent (e.g. virulent pneumococci) they may, in the rabbit at least, be absorbed through the mucosa, appear in the blood-stream, and set up a rapidly fatal infection (Bull and McKee, 1928).

Despite the difficulties and failures in the attempt to produce experimentally pulmonary infections by inhalation or insufflation of organisms under normal conditions—a testimony to the defences of the upper respiratory tract—Blake and Cecil (1920) succeeded in reproducing typical pneumonic lesions in lower monkeys by the intratracheal injection of small quantities of a highly virulent pneumococcus. It seems possible, however, that in their experiments the organisms were not directly aspirated into the lungs, but were absorbed through the injured mucosa into the paratracheal lymphatics and carried thence to the lungs (Winternitz, Smith and Robinson, 1920).

The presence of mechanisms in the upper respiratory tract defensive against the entrance of foreign organisms must be considered in relation to the epidemiology of certain bacterial and virus infections, and to the occurrence of disease-processes such as the common cold, bronchitis and pneumonia. There is a fruitful field for investigation into the precise nature of these defences and how they break down or are overcome so that invading organisms gain a foothold. For example, the work of Kelly (1913) on nasal thermometry has indicated the importance of changes of temperature within the nasal fossæ in relation to pathological conditions therein, and Hill (1919) has demonstrated a reflex turgescence of the nasal mucosa under certain conditions when the feet are chilled. Thus, the occurrence and spread of respiratory infections may be more closely related to an interference with the mechanism of defence than to an enhanced virulence of the infecting organism.

THE GRAM-NEGATIVE COCCI OF THE NASOPHARYNX.

In the examination of smears or cultures from the mucous membrane of the upper respiratory tract of healthy or diseased subjects Gram-negative diplococci are regularly encountered. Morphologically the resemblance to the meningococcus is very close and may present difficulty in the examination of the nasopharynx of meningococcus carriers or

contacts during epidemics of meningitis (see Vol. II, p. 319). In the sputum or in smears from swabs of the throat these organisms appear as typical diplococci, but may also occur as tetrads or in small clumps. In the examination of serum cultures of throat swabs with Neisser's method of staining they are often prominent owing to their peculiar brownish-black staining.

These organisms are practically constant members of the normal flora of the nasopharynx, and are often found in increased numbers in catarrhal affections of the respiratory tract. Some workers have regarded them as possessing some degree of pathogenicity for the human subject and capable of producing symptoms like those of influenza (Ghon and Pfeiffer, 1902 ; Dunn and Gordon, 1905), or as concerned in the common cold. Glynn and Digby (1923), in a study of pneumonia, express the opinion that *M. catarrhalis* may exercise an injurious effect upon the patients. Some of the early workers were of opinion that certain members (*M. catarrhalis* and *M. flavus*) might be found in the spinal fluid of cases of meningitis, but this has not been confirmed. In the examination of sputum for pneumococci by mouse inoculation chromogenic members of the group are often present in considerable numbers. The close resemblance in morphology of these organisms to the meningococcus naturally led the early workers to a careful comparison of the cultural characters of Gram-negative cocci from the nasopharynx. As a result a group of organisms with numerous members was described. Of these *Micrococcus catarrhalis* (Pfeiffer, 1896) was carefully studied by von Lingelsheim (1906) along with *M. pharyngis siccus*, *M. pharyngis cinereus*, *Diplococcus pharyngis flavus* (Types 1, 2 and 3), *Diplococcus crassus* and *Diplococcus mucosus*. Elser and Huntoon (1909) described similar types in the nasopharynx of children with meningitis. At a later date nine different groups, excluding the meningococcus, were described by Gordon (1921) including six different chromogenic strains.

In the separation of the members of the group the appearances of colonies have been regarded as of especial importance, combined with a study of the fermentative activity for various carbohydrates and the production of pigment. The chromogenic characters of some of the members are most easily elicited by culturing on media containing glucose.

Recent workers who have made a detailed study of certain types (Wilson, 1928) or an examination of large numbers of strains (Wilson and Smith, 1928) express the opinion that the criteria for separation of the individual members of the group are insufficient for classification. They found that the study of colony appearances, which was of primary importance in the work of the early investigators, is no longer satisfactory. Numerous variants, rough, smooth, mucoid, or butyrous, may occur, and transformation from one type of colony to another may be observed during prolonged study of a single strain. The fermentation reactions also vary according to the nature of the medium and to the time during

which the reactions are studied, and agreement as to specific types is difficult. Wilson and Smith suggest that for the present all the Gram-negative cocci of the nasopharynx with the exception of the meningococcus should be grouped as *M. pharyngis*. They define *M. pharyngis* as 'non-motile, Gram-negative diplococcus, arranged sometimes in tetrads and often in dense clumps. Grows on agar, giving rise to either smooth or rough colonies, which are generally coherent, tenacious, membranous and friable, are difficult to emulsify and are auto-agglutinable in saline. Grows in serum-broth with the production of little or no turbidity, as a rule, and a coarsely granular sediment not disintegrating completely on shaking; a surface ring growth is not infrequently formed, particularly by the rough variants. A yellow, golden-yellow or greenish-yellow pigment may be produced, but is variable in its appearance. The sugar reactions are subject to variation; glucose, maltose and sucrose may be fermented with the production of acid. Aerobic and will not grow under strictly anaerobic conditions. Growth is best at 37° C. but will generally occur at 23° C. They are non-pathogenic on subcutaneous injection into mice; large doses intraperitoneally may cause death from toxæmia. The group is subject to great variation in colonial appearance and, apart from the smooth and rough types, a smooth variant may occur that is of butyrous consistency and easy to emulsify, and also a mucoid variant containing capsulated diplococci'.

The chromogenic types appear to be almost constantly present, whereas *M. catarrhalis* is uncommon. Wilson, in the course of the examination of swabs from 3,000 persons, samples of the general population, isolated Gram-negative cocci in 95 per cent., relatively few of which were of well-defined types. The meningococcus and *M. catarrhalis* were rarely encountered. Of 85 strains of Gram-negative cocci other than meningococcus and *M. catarrhalis*, collected at random, 67 could be related to one or other of his standard strains and 18 strains showed such diverse characteristics as to prevent their being classified in any of the six groups which he had chosen as standard. A full description of the various types is given by Wilson (1928).

REFERENCES.

- ARNOLD, L., OSTROM, M. L. & SINGER, C., 1928, *Proc. Soc. Exp. Biol.*, N.Y., **25**, 624.
 BLAKE, F. G. & CECIL, R. H., 1920, *J. Exp. Med.*, **31**, 403, 475 and 499.
 BLOOMFIELD, A. L., 1922, *Amer. J. Med. Sci.*, **164**, 854.
 BRANHAM, S. E., 1928, *J. Infect. Dis.*, **42**, 230.
 BULL, C. G. & MCKEE, C. M., 1928, *Amer. J. Hyg.*, **8**, 477 and 723.
 CALAMIDA, U. & BERTARELLI, E., 1902, *Zbl. Bakt.*, Abt. I, Orig., **32**, 428.
 COWDRY, E. V., 1928, *Special Cytology*, New York.
 DUNN, R. A. & GORDON, M. H., 1905, *Brit. Med. J.*, ii, 421.
 ELSEY, W. J. & HUNTOON, F. M., 1909, *J. Med. Res.*, **20**, 371.
 FLEMING, A., 1922, *Proc. Roy. Soc.*, B, **93**, 306.
 GHON, A. & PFEIFFER, H., 1902, *Z. klin. Med.*, **44**, 262.
 GLYNN, E. E. & DIGBY, L., 1923, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 79.
 GORDON, J. E., 1921, *J. Infect. Dis.*, **29**, 462.
 HARVEY, W. C., 1928, *J. Hyg.*, Camb., **28**, 228.
 HILL, L., 1919, *Sp. Rep. Ser. Med. Res. Coun.*, Lond., No. 32.

- JONES, cited from 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, **6**, 455.
- KELLY, A. B., 1913, *J. Laryng.*, Lond., **27**, 515.
- KÜSTER, E. & WÖSSNER, cited from 1913, *Handb. d. path. Mikroorg.* hrsg. Kolle u. Wassermann, **6**, 455.
- VON LINGELSHEIM, W., 1906, *Klin. Jahrb.*, **15**, 373; 1908, *Z. Hyg. InfektKr.*, **59**, 457.
- MCCARTNEY, J. E. & HARVEY, W. C., 1928, *Proc. R. Soc. Med.*, **21**, 845 (Sect. Epidem. & State Med., p. 27).
- NEUMANN R. O., 1902, *Z. Hyg. InfektKr.*, **40**, 33.
- PARK, W. H. & WRIGHT, J., 1898, *J. Laryng.*, Lond., **13**, 247.
- PFEIFFER, H., 1896, vide Flüge, *Die Mikroorganismen*, 3rd Edit., **2**, 154.
- SCHOUSBOE, 1901, cited from 1913, *Handb. d. path. Mikroorg.*, hrsg. Kolle u. Wassermann, **6**, 452.
- THOMSON, ST. CLAIR & HEWLETT, R. T., 1895, *Trans. Med. Chir. Soc.*, **78**, 239; 1896, *Lancet*, Lond., i, 86.
- WHITTLE, C. H., 1928, *J. Hyg.*, Camb., **27**, 200.
- WILSON, S. P., 1928, *J. Path. Bact.*, **31**, 477.
- WILSON, G. S. & SMITH, M. M., 1928, *J. Path. Bact.*, **31**, 597.
- WINTERNITZ, M. C., SMITH, G. H. & ROBINSON, E. S., 1920, *Johns Hopk. Hosp. Bull.*, **31**, 63.
- WURTZ, R. & LERMOYEZ, M., 1893, *C.R. Soc. Biol.*, Paris, 9 ser., **5**, 756.
- YATES, A. L., 1924, *J. Laryng. Otol.*, Lond., **39**, 554.
- ZINSSER, H., 1927, *Text-Book of Bacteriology*, 6th Ed., 454.

The Bacterial Flora of the Normal Gastro-Intestinal Tract.

INTRODUCTION.

The anatomical relationships of the alimentary canal would suggest that bacteria find ready entrance. It has been shown that the mouth contains a heterogeneous flora of aerobic and anaerobic organisms and the food which is ingested, particularly if uncooked, contains numerous viable organisms of many different kinds. The temperature of the interior of the body and the character of the intestinal contents with respect to food material and moisture offer optimum conditions for the growth of many species of organisms. The repeated introduction of fresh materials, the variety of substances used as food, the movements of the contents and the periodic evacuation of food residues and bacterial products create conditions impossible to imitate in artificial cultures, and our knowledge of the conditions under which the bacteria of the intestine live and multiply is extremely limited. The activities of intestinal bacteria have been deduced from the study of pure cultures, but these again may have little relationship to their natural activities under the highly complex conditions of the intestinal canal. Various inhibitory factors also come into play within the intestine, of which the most important is probably the acidity of the gastric juice. The conditions within the canal are obviously favourable for bacterial growth since the fæces contain enormous numbers of organisms. Strasburger (1902) has estimated that a normal individual excretes 8 gm. of bacteria daily, representing a quarter to a third of the fresh fæcal material. Bacteriological examination of the fæces gives little indication of the numbers and variety of bacteria in the upper parts of the alimentary tract, particularly in the small intestine, and the facilities for studying the bacterial flora at various levels of the

intestinal tract of the living human subject are very limited and our knowledge of the subject correspondingly restricted.

It will be convenient to consider first the development of the bacterial flora in the intestine of the infant and the changes which occur in it as the child grows older, then to study the bacteria which occur in the adult, and finally to consider the quantitative and qualitative relationships of the organisms found at different levels of the intestine and the factors which influence these.

DEVELOPMENT OF THE INTESTINAL FLORA.

Billroth first observed that the alimentary tract of newly born babies is sterile and Escherich (1886) noted that the time elapsing before organisms appeared in the dejecta varied with the conditions of the child's environment and that in summer bacteria could be recovered from meconium as early as the fourth hour after birth and in winter not till the twentieth hour. Tissier (1900) found that the first appearance of bacteria in the intestine varied between 10 and 20 hours after birth. Observations by the writers have confirmed these findings, and it may be added that even up to 30 hours after birth bacteria in the meconium are scanty. This *aseptic phase*, as Tissier called it, is followed by one of *mixed infection* when the intestine becomes invaded by a promiscuous collection of organisms representative of the bacteria found in the child's surroundings. The organisms which are first demonstrable in direct smear and on culture are Gram-positive cocci (*Staphylococcus albus* and enterococci) followed by Gram-negative coliform bacilli and spore-bearing organisms, a terminal sporing organism, the 'köpfchensporen' of the German writers, identified as the *Bacillus putrificus* of Bienstock, being often present. With the appearance of faecal material organisms become more abundant, and slender, straight or slightly curved Gram-positive bacilli make their appearance, representing the establishment in the intestine of the aciduric organism *B. bifidus*. From the fourth or fifth day the heterogeneous collection of bacteria is gradually supplanted by *B. bifidus* so that the intestinal flora of a breast-fed infant 7 to 8 days old is composed almost entirely of slender non-sporing Gram-positive bacilli. This flora persists with little variation so long as the child is breast-fed. During that period *B. bifidus* constitutes 90 to 95 per cent. of the bacteria in the faeces. Although coliform organisms and enterococci (*S. faecalis*) may not be evident in the direct smear of faeces stained by Gram's method, their presence can usually be demonstrated by cultural methods. Spore-bearing organisms are not commonly present in the intestinal flora of the breast-fed child.

The almost homogeneous flora of the suckling is in striking contrast to that of the artificially fed infant. Films made directly from the faeces of the latter show a mixed flora in which are represented Gram-negative coliform organisms, Gram-positive bacilli and cocci, and less numerous spore-bearing organisms and Gram-negative cocci. There is no constant

proportion of any of these types ; for example, in a series of 30 babies in whom counts were made from a direct smear of the fæces stained by Gram's method, the coliform organisms varied from 30 to 80 per cent. of the total, and the Gram-positive bacilli of aciduric type from 20 to 70 per cent. In addition, spore-forming organisms are usually present in the fæces of the artificially fed child, the types most commonly isolated being *B. welchii*, *B. sporogenes* and *B. putrificus*.

As the age of the child increases and the diet becomes more varied, there is an increasing variety of organisms in the fæces. Coliform organisms now form a larger proportion of the flora and the aciduric group is only sparsely represented, while spore-bearing organisms are numerous and Gram-positive cocci are always present. There is no constant or typical flora—as seen in the examination of the fæces—for the child after the first year of life or for the adult. Numerous factors contribute to alter the fæcal flora and there are wide variations in the normal, so that it is difficult to identify an ' abnormal flora ' by the methods of examination at present available. A gross numerical increase of a particular organism or of the organisms of a group (for example, *B. welchii*, the coliform organisms or the streptococci) over the average normal count may be demonstrated by the method of successive dilution and has been shown to occur in pernicious anæmia (Torrey and Kahn, 1923, Davidson, 1928). Despite the variability of the fæcal flora and its susceptibility to change under varying conditions, there is increasing evidence that in the individual, and possibly in the species, the intestine harbours an indigenous or ' native ' flora and that only a small proportion of the bacteria which are ingested are capable of establishing themselves within the gut or on its healthy mucosa.

DIVISION OF INTESTINAL FLORA INTO DIFFERENT GROUPS.

Many different organisms are present in the fæces, and cultural methods such as are commonly employed fail to demonstrate many of the bacteria which may be seen in an examination of the direct smear. It is probably true that many fæcal bacteria are dead, but many organisms, which are not otherwise recoverable, may be isolated by the use of special media and methods. Classification of the intestinal organisms may be made either according to morphological and cultural characters or according to their biochemical reactions. A morphological classification, e.g. that suggested by Logan (1914), recognizes four main groups of organisms, (a) the Gram-negative coliform group, (b) the aciduric group of Gram-positive bacilli, (c) the Gram-positive cocci, and (d) the spore-bearing organisms—aerobic and anaerobic. These groups do not represent all the types present in the intestine for they do not include the Gram-negative cocci, spirilla, spirochætes, yeasts and moulds, but they constitute the bulk of the intestinal organisms. A full description of the methods and media used for the isolation of the different groups may be found in the papers of Logan (1914), Morris, Porter and Meyer (1919) and Davidson (1928).

The Coliform Group.

The Gram-negative coliform bacilli form the major proportion of the faecal flora of the adult. Only in the intestine of the breast-fed infant are they scantily represented. The types most commonly present are *B. acidilactici*, *B. coli communis* and *communior* or variants of these. *B. lactis aerogenes* is stated by some writers to be a common inhabitant of the upper intestine, but by the ordinary methods of isolation this organism is not often recovered from the faeces of healthy persons. Bamforth (1928) reported its presence in only 32 of 976 specimens of faeces examined by him, and these mostly from cases of enteritis or dysentery. It is, however, worthy of note that *B. lactis aerogenes* is not infrequently found in infections of the urinary tract in the human subject, and is frequently found in large numbers in the faeces of children suffering from intestinal disorders.

Recent work by one of the writers using the synthetic citrate medium of Koser (1924) for primary culture of the stool and subsequent plating on citrate agar has shown that *B. lactis aerogenes*, *B. cloacæ* and other organisms of the same group can be isolated from the stools of the majority of normal people although they are present only in small numbers. The same method also serves to isolate *B. pyocyaneus*, varieties of paracolon bacilli, *B. proteus*, *Morgan's bacillus* and various unnamed members of the coliform group. A greater diversity of types of organisms of the coliform group can be demonstrated in faeces by this method than by the ordinary methods of culture. The use of fluid media of different hydrogen-ion concentrations for primary culture or of substances which inhibit the growth of certain strains may also reveal types of coliform organisms which are not ordinarily encountered. For example, Browning, Gilmour and Mackie (1913) found that the addition of brilliant green to peptone water inhibits the growth of *B. coli* and that inositol-fermenting organisms may be isolated in this way when they are not demonstrable by direct plating. The types of coliform organisms (identified by biochemical reactions and by serological methods) which may be isolated from the faeces of an individual remain constant over a prolonged period, but a change in the conditions within the intestine such as occurs with the use of purgatives or in diarrhoeal conditions may be followed by the presence in the faeces of types of coliform organisms which are not usually encountered. The explanation may be that an altered reaction in the intestine favours the proliferation of types which are normally only scantily present, or that these organisms are living at a higher level in the intestine, and are rapidly carried into the large intestine by the increased rate of peristalsis.

Attempts have been made to differentiate intestinal from soil coliform organisms on the basis of certain chemical reactions [indole and acetyl-methyl-carbinol formation, reduction of neutral red and ability to utilize sodium citrate in a synthetic medium (Koser, 1924)]. The majority of the coliform organisms commonly isolated from the faeces form indole,

do not produce acetyl-methyl-carbinol from sugars and do not grow in Koser's synthetic medium, while the opposite is true for the soil coliform organisms. Since organisms, such as *B. lactis aerogenes*, which, according to these tests would be classified as soil bacteria, can be recovered from the fæces of the majority of healthy persons the distinction is not reliable. Similarly, attempts to separate the coliform bacteria of warm-blooded animals from those present in the intestine of cold-blooded animals and in the soil by the test of heat-stability (fermentation of glucose at 46° C.) have not been wholly successful.

The Aciduric Group of Gram-positive Bacilli.

Gram-positive non-sporing bacilli form the bulk of the bacterial flora in the fæces of the suckling. They are less numerous in the stools of the artificially fed child, and in adults they may be only sparsely present in a smear made directly from the fæces. The difficulty of isolating these Gram-positive bacilli by the ordinary cultural methods led to the assumption that they were dead bacteria until the researches of Tissier (1900), Moro (1900), and Finkelstein (1900) showed that they could be recovered by the use of media in which a degree of acidity was developed sufficient to destroy most of the other intestinal bacteria. A description of the methods in use for the isolation of these acid-resistant—or aciduric—bacilli and the cultural characters of the principal members of the group—*B. bifidus* and *B. acidophilus*—is given at page 369. *B. bifidus*, which is more micro-aerophilic than *B. acidophilus*, is the predominant organism in the intestine of the breast-fed infant, whereas *B. acidophilus* is the type present in the fæces of older children and adults. The diminishing proportion of aciduric organisms in the intestinal flora from infancy to adult life is probably largely dependent upon changes in the dietary.

The Gram-positive Cocci.

The Gram-positive diplococci of the intestine belong, according to the classification of Smith and Brown (1914), to the non-hæmolytic 'gamma' group of streptococci. Considerable pleomorphism in the group and the consequent labelling of them under different names such as *Enterococcus* (Thiercelin, 1899), *Micrococcus ovalis* (Hirsch and Libman, 1897) and *Streptococcus faecalis* (Andrewes and Horder, 1906) has led to confusion in the identification of the intestinal streptococci. Dible's studies (1921) have shown that there are numerous variants from the most common type, which is characterized by its heat-resistance, short chain formation and mannitol fermentation. They also exhibit a tolerance towards extremely acid and alkaline cultural conditions (Downie and Cruickshank, 1928). The property of heat-resistance or of survival in strongly alkaline media may be utilized for the isolation of fæcal streptococci and the demonstration of their invariable presence in the fæces of healthy individuals. The relationship of the intestinal streptococci to the streptococci of the mouth and throat has not been thoroughly investigated.

Streptococci of faecal type may be isolated from the throats of healthy persons by the alkaline technique. The appearance of unusually large numbers of streptococci in the faeces has been interpreted as an indication of a pathological condition in the intestine predisposing to diseased conditions elsewhere in the body, e.g. rheumatoid arthritis, but it is more reasonable to regard such findings as evidence of temporary derangement of intestinal function or the result of alteration in the diet, since it is known that excessive amounts of carbohydrate favour the proliferation of these bacteria.

Spore-bearing Bacteria, Aerobic and Anaerobic.

The aerobic spore-bearing organisms present in the intestine have not received much attention. Considerable numbers of these must be ingested with food, and recent investigations have shown that they are not infrequently present in the upper part of the small intestine (Goldman, 1924). The types most commonly identified are *B. mesentericus*, *B. megatherium*, *B. mycoides* and *B. subtilis*, and these strongly proteolytic organisms may play some part in the breakdown of undigested food residue. The study of the anaerobic spore-bearing organisms in the intestine requires special methods and much patience. Repeated subculture after isolation and preferably the use of the Barber single-cell technique is necessary to ensure purity of the strains. Among a large variety of anaerobes which may be present in the intestine *B. welchii* and *B. sporogenes* are probably the most common. *B. histolyticus*, *B. putrificus*, *B. tertius*, *B. tetanoides* and *B. tetani* itself as well as other less well-known types, have all been isolated from the faeces of healthy persons. *B. tetani* is present more often in the intestines of animals such as horse, cow, sheep, &c., but TenBroeck and Bauer (1922) recovered toxic strains from 37·7 per cent. of a Chinese population. Fildes (1925) and Kerrin (1928, 1929) in this country, on the other hand, have found that the organism is very seldom present in the faeces of human cases.

Spirochaetes.

The presence in the faeces of spirillar and spirochaete forms has been described by many observers, particularly in conditions of the large intestine associated with severe diarrhoea. In cholera, dysentery, enterocolitis and other severe infections of the intestinal tract large numbers of these forms may be demonstrated in the faeces, and it has been suggested that pathogenic forms occur. Numerous varieties of these organisms have been described, and it would appear that the caecum and ascending colon afford conditions suitable for their development and that they are not merely temporary invaders. In a large series of normal individuals examined by Parr (1923) spirochaetes were demonstrated in about one-third of the cases, and were found to be constantly present over a period of several months. Owing to the difficulty of staining and of obtaining pure cultures it is not possible to say what relationship, if any,

these intestinal spirochætes have to spirochætes found in other parts of the body. The subject of spirochætes in the cæcum has been recently discussed by Sanarelli (1927), Delamare (1924) and Sáenz (1925).

Moulds and Yeasts.

Many varieties of yeast-like fungi are present in the fæces and have been isolated from the stomach and intestines of normal people. The majority of the strains have been placed on morphological and cultural characters in the group Para-saccharomyces A, and the members cannot be distinguished from named strains of *Monilia psilosis* (Nye, Zerfas and Cornwell, 1928). In gastric achylia they may be found in large numbers in the gastric contents. The results suggest that the finding of *Monilia psilosis* in the stools in sprue may have no ætiological significance.

An Alternative Method of Classification.

An alternative method of classifying intestinal organisms which is of value in relation to clinical conditions is the division of the bacteria according to their katabolic activities on protein and carbohydrate; there are accordingly two main groups, proteolytic and saccharolytic. In addition, some bacteria have the power of breaking down both carbohydrate and protein; generally speaking, these utilize any available carbohydrate before attacking protein. According as saccharolytic or proteolytic organisms predominate, the fæcal flora is called saccharolytic (fermentative) or proteolytic (putrefactive). The important saccharolytic organisms in the intestine are *B. bifidus*, *B. acidophilus*, the *enterococcus*, *B. welchii* and *B. tertius*. The conditions which favour the growth of these organisms may particularly suit the development of *B. bifidus* and *B. acidophilus*—the acid-resistant organisms—and an 'aciduric' flora may result, the best example of which is found in the suckling infant's intestine. The proteolytic group includes *B. sporogenes*, *B. putrificus*, *B. mesentericus*: *B. proteus*, *B. pyocyaneus* and certain anaerobic spore-bearing organisms have both saccharolytic and proteolytic powers. Most of the proteolytic bacteria may be isolated from the fæces of apparently healthy persons, and it has been suggested that the term 'facultatively proteolytic' should be applied to the normal intestinal flora of the child or adult to distinguish it from a putrefactive, and possibly harmful, flora which is defined by the marked proteolysis produced by its constituent members on certain media (Morris, Porter and Meyer, 1919). The difference between a facultative and a putrefactive flora is only one of degree.

THE BACTERIAL FLORA AT DIFFERENT LEVELS OF THE INTESTINE OF THE ADULT.

Examination of the fæces affords little information about the bacterial flora in the stomach or in the higher levels of the intestine, and various methods have been devised for the study of these regions. In experimental animals loops of the bowel at different levels may be isolated and their

contents examined immediately after death. In the human subject the examination of fistulas, accidentally or specifically produced, or of isolated loops of intestine has yielded information about the bacterial flora at various levels, but the results have not been generally accepted, the conditions being regarded as abnormal.

Arnold (1926) recommends ventral fixation of the small intestine of dogs without interfering with the continuity of the bowel as a preliminary to the abstraction of samples of the contents. The withdrawal of stomach or intestinal contents by means of a tube passed from the mouth into the stomach and through the pylorus to the small intestine has been used in recent years for bacteriological examinations, first by Hess (1912) for the duodenum of children, and for the small intestine by Ganter and van der Reis (1921, 1922). It is claimed that levels of the intestine as far distant as 250 cm. from the pylorus may be investigated (Bogendorfer, 1922).

Stomach. Smears made from the gastric contents during digestion show a large variety of organisms, Gram-positive cocci, yeasts and sarcinæ, sporing bacteria, vibrios, spirochætes and leptothrix-like organisms. With the exception of the spore-bearing aerobes (*B. mesentericus*, *B. megatherium*, *B. subtilis*), which are probably carried in with food, the bacteria are largely derived from the mouth. Coliform organisms are rarely isolated and are usually of *B. lactis aerogenes* type. *B. welchii* and *B. sporogenes* may be found along with lactic acid bacilli (Boas-Oppler bacillus) when there is delay in emptying the stomach. Three to four hours after a meal the gastric contents are practically sterile.

Duodenum. The rapid transit of the food-chyme and the acid reaction of the contents (McNeal and Chase, 1913) with possibly bactericidal activity of the digestive secretions or of the intestinal mucosa—although that is denied by Kendall and his co-workers (1927)—appear to render the duodenum an unfavourable site for the proliferation of organisms. Consequently cultures from the duodenum are sterile or yield only scanty numbers of bacteria; for example, Kendall found that 26 of 50 normal adults had few or no bacteria in their duodenal contents. The predominant organisms are enterococci, staphylococci, sarcinæ, and, less frequently, coliform organisms of *B. lactis aerogenes* type. Gram-positive aciduric bacilli have also been recovered. Sporing organisms are usually absent.

Jejunum. The bacterial flora of the jejunum at a distance of 25 to 80 inches from the pylorus is still scanty and consists of Gram-negative coliform bacilli, gamma type streptococci, *B. acidophilus* and spore-bearing organisms, the last apparently associated with meat in the diet, since they were absent in patients on a light hospital diet (Goldman, 1924). The presence of an obligate flora is suggested by the fact that several specimens from one individual may show the same types of organisms on each occasion and in approximately similar numbers (Goldman, 1924), while the general bacterial picture in the examination of 350 cases, according to van der Reis (1922), was essentially the same. The number

of viable organisms may be considerably higher in patients with gastro-intestinal symptoms, or with certain diseases, such as rheumatoid arthritis, whose genesis has been associated with intestinal infection (Goldman).

Ileum. With increasing distance from the pylorus bacteria become more abundant, and from the examination of dogs it would seem that enterococci form a much smaller proportion of the total organisms, while coliform organisms become more numerous and varied in type. *B. acidophilus* may be present in small numbers and spore-bearing aerobes and anaerobes in greater.

Cæcum and colon. The diminution of peristalsis in the cæcum and colon, the increased consistency of the food-residue and a reaction approaching neutrality afford optimum conditions for the proliferation of bacteria. It is probable that in the cæcum and ascending colon bacterial life is more vigorous than elsewhere in the body. In the further passage along the large intestine owing to the abstraction of water the conditions become less favourable, many organisms die, while others change from the vegetative to the spore state.

FACTORS WHICH INFLUENCE THE CHARACTERS OF THE INTESTINAL FLORA.

The factors to which importance may be attributed in influencing the character and numbers of the intestinal bacteria are (*a*) the acid secretion of the stomach, (*b*) the reaction of the intestinal contents, (*c*) the chemical nature of the food and (*d*) the antagonistic action of the normal indigenous flora to the entry of foreign bacteria and the possible bactericidal action of the intestinal mucosa.

The acid-secretion of the stomach. Although bacteria in great variety and numbers are taken into the stomach with food or swallowed from the mouth and nasopharynx the gastric contents some hours after a meal are practically sterile. The reaction in a stomach secreting a normal amount of hydrochloric acid quickly reaches a degree of acidity which is lethal to all but the hardy and acid-resistant organisms such as yeasts, sarcinæ and the lactic acid bacilli. In conditions of hypochlorhydria the sterilization is less efficient and in achlorhydria is almost absent. In pernicious anæmia, therefore, and in other conditions where the secretion of HCl is meagre or absent, the bacterial flora of the stomach and adjacent part of the small intestine as well as that of the fæces differs, at least quantitatively, from that in health. During normal digestion, although the majority of organisms are killed, some are undoubtedly carried into the intestine with the food, and it is probable that in the intervals of digestion or in the drinking of fluids organisms from the mouth may find an easy passage directly into the intestine through the relaxed pyloric sphincter.

Reaction of the chyme. The reaction of the intestinal contents undergoes considerable change in its passage along the bowel. In the duodenum the reaction is dependent largely on the secretion of the stomach, and is, as a rule, distinctly acid, so that bacterial life is inhibited. Arnold (1928)

found the hydrogen-ion concentration of the duodenal contents to vary between pH 5.2 and 6.0 with limits of pH 4.0 to 8.0. As the chyme passes along the bowel the reaction changes owing to admixture with bile, pancreatic juice and succus entericus and becomes more favourable to bacterial growth. The cæcum and colon provide the intestinal organisms with optimum conditions for their proliferation, and in the absence of excess of fermentable carbohydrate the reaction becomes neutral or alkaline (Robinson, 1922), and the flora comes to resemble that of the fæces.

Nature and quantity of food. Although attempts to control putrefactive activity in the gut were made as early as 1886 (Hirschler, 1886), Herter and Kendall (1909) were probably the first workers to examine carefully the relationship of the chemical characters of the food to the intestinal flora. From their experiments on cats and monkeys they concluded that a high carbohydrate diet tends to eliminate 'putrefactive' bacteria from the intestine and to substitute instead a 'fermentative' flora. It has since been shown that the intestinal flora of animals and of man may be considerably altered by the ingestion of certain food-stuffs (Torrey, 1919; Rettger and Cheplin, 1921; Cruickshank, 1928). Lactose, dextrin and starchy foods are especially effective in changing the flora to a saccharolytic or aciduric type. Animal proteins in large amount produce a proteolytic flora whereas fats and vegetable proteins do not affect the intestinal flora (Torrey).

The explanation of the effect of diets rich in lactose and dextrin is not clear. Kendall has suggested that as these substances are absorbed relatively slowly when given in the large amounts required to transform the flora they reach the cæcum and colon unchanged and afford readily utilizable material for the growth of aciduric organisms. Laboratory experiments have shown that in mixed cultures of *B. acidophilus*, *B. coli* and spore-bearing aerobes in a carbohydrate medium, *B. acidophilus* is alone capable of surviving the acid conditions which are created (Cruickshank, 1925; Torrey and Kahn, 1923). It has been found that the reaction of the fæces when an aciduric flora has been produced does not differ from normal, although Robinson (1922) has pointed out that the contents may be acid in the cæcum and are changed to the normal reaction during passage along the large intestine. As the saccharolytic activity of *B. acidophilus* is not very high as compared with many other intestinal organisms, e.g. *B. coli*, it would seem more probable that the carbohydrate is first attacked by these organisms and that the conditions which result are favourable for the development of *B. acidophilus*. It may be noted that *B. coli* and *B. acidophilus* are to some extent antagonistic since it is usually necessary in isolating *B. acidophilus* from fæces to take measures to prevent the growth of *B. coli*. Experimental work with filtrates of cultures of *B. coli*, enterococci and *B. bifidus* have thrown little light on this subject (Upton, 1929). The biochemical activities of intestinal organisms at different levels are at present a matter for conjecture and

further work on the relationship of the products of carbohydrate metabolism of one organism on the growth of others seems to be required. In breast-fed infants the stools are acid and the flora aciduric, but in diarrhoeal conditions also the reaction of the fæces is usually acid, without, however, any indication of increase in aciduric organisms. Increased peristaltic action alone may play an important part in altering the character of the faecal flora.

There is evidence that fasting reduces the numbers of bacteria in the higher levels of the intestine (Sisson, 1917; Cushing and Livingood, 1900), and it has been observed that patients on a light meat-free diet do not harbour spore-bearing organisms in the jejunum.

The autodisinfecting power of the intestinal mucosa. Attempts to introduce into the bowel bacteria not normally found in the intestine have usually failed. Even when organisms escape the sterilizing mechanism of the stomach or are carried safely past it in capsules, they are not recovered from the fæces or are present only so long as the organism is being given in very large amounts. They pass through the intestine like inert material and fail to 'implant' themselves in the contents. It has been suggested that there exists in the intestine some disinfecting mechanism which allows the obligate or indigenous flora to flourish but kills or inhibits the growth of foreign invaders. Experiments *in vitro* have failed to prove that any single factor or combination of factors, such as the digestive juices or the intestinal mucosa, is responsible for the bactericidal action (Rolly and Leibermeister, 1905). Ganter and van der Reis showed that *B. prodigiosus* was destroyed if introduced into the small intestine, but could be recovered from the fæces if implanted in the large intestine. These experiments have suggested that the intestinal epithelium normally exerts a bactericidal action on 'foreign' bacteria.

The isolation of bacteriophage from the fæces of individuals not suffering from intestinal derangement is suggestive that a bacteriolytic agent apart from the intestinal mucosa may play a part in maintaining the 'native' flora of the bowel, only such organisms as are habituated or resistant to its action being allowed to live and multiply. On the other hand, the recovery of a lytic agent for *B. paratyphosus* B. from the fæces of a chronic paratyphoid carrier at the time that the pathogenic organism was also isolated does not suggest that bacteriophage is an important factor in the regulation of organismal life in the intestine.

THE FUNCTION OF THE INTESTINAL BACTERIA.

Since Pasteur stated his belief that life would not be possible without intestinal bacteria, experiments have been carried out to test the possibility of rearing animals with germ-free intestines. The evidence suggests that the bacteria of the intestine are not essential to life but that they may fulfil certain beneficial functions. They are capable of breaking down food residues and indigestible material to simpler products which may be absorbed and utilized, and in herbivora, the intestinal bacteria, including

moulds and fungi, break down and render assimilable as food, cellulose and cellulose-enclosed starch. They may also help in the destruction of harmful foreign organisms either by a directly antagonistic action to these bacteria or by the production of substances which inhibit their development. Attempts to implant in the intestine of animals or man innocuous non-indigenous organisms such as *B. prodigiosus* usually fail. An example of antagonistic action is indicated by the tendency for typhoid bacilli to become less numerous in their passage along the intestine in certain chronic typhoid carriers. In-vitro experiments showed that this inhibitory effect upon *B. typhosus* was a function of certain strains of *B. coli* isolated from the fæces of typhoid carriers (Smith, 1924). The beneficial effect of an acid reaction in the intestine, apparently due to bacterial action upon available carbohydrate, is shown by the relative immunity to acute enteritis of breast-fed infants whose intestinal contents are acid and whose flora is aciduric. Many attempts have been made to suppress the growth of putrefactive organisms in the intestine by the implantation of acid-producing bacteria: Escherich used *B. lactis aerogenes*; Quincke, yeasts; Metchnikoff, *Lacto-bacillus bulgaricus*; and many workers have used *Lacto-bacillus acidophilus* in the treatment of constipation and of conditions suggesting intestinal toxæmia.

Much attention has been given to the potentiality for harm of the intestinal bacteria and a wide variety of diseases has been attributed to the production and absorption of toxic bodies elaborated in the intestine. The subject has been fully reviewed by Alvarez (1924) and the conclusion seems justified that there is no proof either on clinical or experimental grounds for this belief. The intact mucous membrane of the bowel seems to be an effective barrier to the absorption of toxic products, and, according to some workers, mucus is the all-important defence against the invasion of bacteria and their toxins; its protective action is apparently biochemical as well as mechanical. The effect which deficiency of certain vitamins and especially vitamin A has upon the defence-mechanism of the mucus cells of the intestine has been studied by several workers (Cramer, 1923; Green and Mellanby, 1928), who have shown that under experimental conditions deficiency in vitamin A increases the permeability of the mucosa and permits the entry into the body of organisms which normally are held at bay. It seems, however, that even in health there may be the passage of bacteria from the intestine to the mesenteric glands and other organs of the body (Arnold, 1926). Adami (1914) has applied the term 'latent infection' or 'subinfection' to a condition in which bacteria of low virulence derived from the bowel multiply in the tissues or organs of the body so that chronic or acute infections readily supervene.

In acute intestinal obstruction there is evidence both clinical and experimental of the formation of toxic substances within the bowel (Dragstedt, Cannon and Dragstedt, 1922), and it is commonly thought that the resulting toxæmia is wholly due to the absorption of harmful products formed in the bowel above the site of obstruction. However,

other work suggests that the symptoms may be in part the result of disturbances in the intestine below the site of obstruction (Saito and Saito *et al.*, 1927). In a recent experimental and clinical study of acute intestinal obstruction Williams (1926) expresses the opinion that the toxin of *B. welchii* plays a predominant part in the symptomatology and he claims good results clinically from the treatment of such cases with *B. welchii* antitoxin.

It is more difficult to assess the part played by the intestinal bacteria in the pathogenesis of pernicious anæmia or to determine the relation of the intestinal flora to the altered conditions which exist within the intestine in rickets and other deficiency diseases. The view that hæmolytic bacteria in the alimentary canal are causally related to pernicious anæmia has been held since William Hunter advanced the theory that glossitis and other manifestations of the disease were evidence of bacterial infection, probably streptococcal. Hæmolytic *B. coli*, the streptococcus and *B. welchii* have each been suggested as the causative organism. Hæmolytic streptococci and hæmolytic coliform bacilli are rarely present in the intestine, but there is usually in pernicious anæmia a great numerical increase in the fæces of *B. welchii*, non-hæmolytic streptococci and *B. coli*, and an encroachment by these organisms upon the higher levels of the intestine (Moench, Kahn and Torrey, 1925; Davidson, 1928). There is no evidence that toxins produced by these organisms are directly responsible for the hæmolytic anæmia. The encroachment of bacteria in the higher levels of the intestine may predispose to the absorption of toxic products which damage the liver and prevent the formation by the liver of the substance which, according to Minot and Murphy, is essential to the proper maturation of the megaloblasts. The intestinal flora may thus indirectly play a part in the ætiology of pernicious anæmia.

The pathogenesis of rickets is still unsettled, but there is no evidence that intestinal bacteria play a part in the ætiology, although various workers have noted changes in the reaction of the intestinal contents and fæces of animals in which experimental rickets or other deficiency diseases had been produced. It seems probable that such alterations of reaction, usually to the alkaline side, are directly dependent on the dietary (e.g. the MacCollum diet is particularly rich in calcium) and have no relation to the character of the bacterial flora which is present.

REFERENCES.

- ADAMI, J. G., 1914, *Brit. Med. J.*, i, 177.
 ALVAREZ, W. C., 1924, *Physiol. Rev.*, 4, 352.
 ANDREWES, F. W. & HORDER, T. J., 1906, *Lancet*, Lond., ii, 708.
 ARNOLD, L., 1926, *J. Infect. Dis.*, 38, 246; 1928, *Amer. J. Hyg.*, 8, 604.
 BAMFORTH, J., 1928, *J. Hyg.*, Camb., 27, 343.
 BOGENDORFER, R., 1922, *Deuts. Arch. klin. Med.*, 140, 257.
 BROWN, J. H., 1919, *Monogs. Rockefeller Inst. Med. Res.*, 9.
 BROWNING, C. H., GILMOUR, W. & MACKIE, T. J., 1913, *J. Path. Bact.*, 18, 146.
 CRAMER, W., 1923, *Lancet*, Lond., i, 1046; 1924, *ibid.*, i, 633.

- CRUICKSHANK, R., 1925, *J. Hyg.*, Camb., **24**, 241; 1928, *Brit. J. Exp. Path.*, **9**, 318.
- CUSHING, H. & LIVINGOOD, 1900, *Contrib. Sc. Med. Pupils W. H. Welch*, p. 543.
- DAVIDSON, L. S. P., 1928, *J. Path. Bact.*, **31**, 557.
- DELAMARE, G., 1924, *Ann. Méd.*, **15**, 315.
- DIBLE, J. H., 1921, *J. Path. Bact.*, **24**, 3.
- DOWNIE, A. W. & CRUICKSHANK, J., 1928, *Brit. J. Exp. Path.*, **9**, 171.
- DRAGSTEDT, L. R., CANNON, P. R. & DRAGSTEDT, C. A., 1922, *J. Infect. Dis.*, **31**, 209.
- ESCHERICH, L. S., 1886, *Münch. med. Wschr.*, **33**, 43.
- FILDES, P., 1925, *Brit. J. Exp. Path.*, **6**, 62.
- FINKELSTEIN, H., 1900, *Deuts. med. Wschr.*, **26**, 263.
- GANTER & VAN DER REIS, 1921, *Deuts. Arch. klin. Med.*, **137**, 348; 1922, *Münch. med. Wschr.*, **69**, 347.
- GOLDMAN, A., 1924, *J. Infect. Dis.*, **34**, 459.
- GREEN, H. N. & MELLANBY, E., 1928, *Brit. Med. J.*, ii, 691.
- HERTER, C. A. & KENDALL, A. I., 1909, *J. Biol. Chem.*, **7**, 203.
- HESS, A. F., 1912, *Amer. J. Dis. Child.*, **3**, 304.
- HIRSCH & LIBMAN, E., 1897, *Zbl. Bakt.*, **33**, 369 and 376.
- HIRSCHLER, A., 1886, *Hoppe-Seyl. Z.*, **10**, 306.
- KENDALL, A. I., 1911, *J. Med. Res.*, **25**, 117.
- KENDALL, A. I., DAY, A. A., WALKER, A. W. & HANER, R. C., 1927, *J. Infect. Dis.*, **40**, 677.
- KERRIN, J. C., 1928, *Brit. J. Exp. Path.*, **9**, 69; 1929, *ibid.*, **10**, 370.
- KOSER, S. A., 1924, *J. Bact.*, **9**, 59.
- LOGAN, W. R., 1914, *J. Path. Bact.*, **18**, 527.
- MCNEAL & CHASE, 1913, *Arch. Intern. Med.*, **12**, 178.
- MOENCH, L. M., KAHN, M. C. & TORREY, J. C., 1925, *J. Infect. Dis.*, **37**, 161.
- MORO, E., 1900, *Wien. klin. Wschr.*, **13**, 114.
- MORRIS, G. B., PORTER, R. L. & MEYER, K. F., 1919, *J. Infect. Dis.*, **25**, 349.
- NYE, R. N., ZERFAS, L. G. & CORNWELL, M. A., 1928, *Amer. J. Med. Sci.*, **175**, 153.
- PARR, L. W., 1923, *J. Infect. Dis.*, **33**, 369.
- VAN DER REIS, V., 1922, *Klin. Wschr.*, **1**, 950.
- RETTGER, L. F. & CHEPLIN, H. A., 1921, *The Transformation of the Intestinal Flora*, Newhaven and London.
- ROBINSON, C. S., 1922, *J. Biol. Chem.*, **52**, 445.
- ROLLY & LEIBERMEISTER, G., 1905, *Deuts. Arch. klin. Med.*, **83**, 413.
- SÄENZ, A., 1925, *An. Fac. Med. Montevideo*, **10**, 621.
- SAITO, S., 1927, *Jap. J. Med. Sci.*, **1**, 1.
- SAITO, S., SAKAI, K. & SUZUKI, S., 1927, *Jap. J. Med. Sci.*, **1**, 27.
- SANARELLI, G., 1927, *Ann. Inst. Pasteur*, **41**, 1.
- SISSON, W. R., 1917, *Amer. J. Dis. Child.*, **13**, 117.
- SMITH, R. P., 1924, *Brit. Med. J.*, ii, 948.
- SMITH, T. & BROWN, J. H., 1914, *J. Med. Res.*, **31**, 455.
- STRASBURGER, J., 1902, *Z. klin. Med.*, **46**, 413.
- TENBROECK, C. & BAUER, J. H., 1922, *J. Exp. Med.*, **36**, 261.
- THIERCELIN, E., 1899, *C.R. Soc. Biol.*, Paris, 10th ser., **5**, 269.
- TISSIER, H., 1900, *La Flore Intestinale chez le Nourrisson*, Paris.
- TORREY, J. C., 1919, *J. Med. Res.*, **39**, 415.
- TORREY, J. C. & KAHN, M. C., 1923, *J. Infect. Dis.*, **33**, 482.
- UPTON, M., 1929, *J. Bact.*, **17**, 315.
- WILLIAMS, B. W., 1926, *Brit. J. Surg.*, **14**, 295.

The Bacterial Flora of the Vagina.

The early studies of the bacterial flora of the external genitals and vagina were directed to the detection of pathogenic organisms whose presence in these situations might explain the occurrence of septic infections following childbirth. In 1887 Goenner stated that the vaginal secretion of pregnant women did not contain the bacteria usually found in cases of puerperal infection and that although organisms were present in

abundance these did not grow on ordinary culture media. In 1892 Döderlein's monograph on the vaginal secretion of pregnant women appeared, in which he described the bacillus to which his name is attached.

The most striking feature of Döderlein's work was the demonstration that the vaginal secretion of many pregnant women shows an extremely simple, almost homogeneous bacillary flora. These findings were corroborated by Krönig (1894) and others. The marked contrast between the simplicity of the bacterial flora of the vagina and the heterogeneous collection of organisms of the external genitals led to the assumption that the vaginal secretions possessed bactericidal powers, and much experimental work was carried out in an attempt to demonstrate this property. Döderlein, in the course of his study of the cultural characters of the vaginal bacillus which he isolated, had found that considerable quantities of lactic acid were formed by it and that in mixed cultures with staphylococci, the latter were quickly killed by the resulting acid condition. He attributed the bactericidal mechanism of the vaginal secretion in pregnancy to the acid produced in the secretion by the growth of the vaginal bacillus. In the examination of a series of women he found that nearly 50 per cent. showed a highly acid vaginal secretion containing large numbers of the vaginal bacillus, some yeasts, and practically no other organisms. He regarded these cases as normal. In the other cases the secretion was slightly acid, neutral, or alkaline, and exhibited a mixed flora with absence of the vaginal bacillus. He was of opinion that in this pathological group pathogenic organisms were present, and, as a result of the study of a further series of cases which he followed during the puerperium, that there was a greater incidence of puerperal illness in the pathological group. Investigation by other workers corroborated Döderlein's general findings, but there was much difference of opinion regarding the presence of pathogenic organisms. It was shown by Menge and others that the property of eliminating organisms artificially introduced into the vaginal canal was independent of the acidity of the vaginal secretion, as similar self-cleansing power was present in women whose secretions were neutral or alkaline in reaction. According to Krönig the vagina of newly born children and virgins showed a similar power of disinfection. In experiments on animals in which the vaginal fluid is always alkaline Cahanesco (1901) found that the self-cleansing power was relatively feeble and differed from animal to animal and from organism to organism. He expressed the opinion that the direction of the current and the continual epithelial desquamation mechanically carried organisms to the vaginal outlet and that leucocytosis was of importance only after the artificial introduction of large numbers of organisms. In experiments *in vitro* he was unable to demonstrate any antagonism between the secretions and various common organisms.

The marked acidity of the vaginal secretions of most adult women has been noted by all observers. During pregnancy the acidity rises markedly and is at its highest point just before parturition (Cruickshank and

Baird, 1930). The acidity is due to lactic acid, which is present in a concentration of 0.4 per cent. (Haag, 1915; Becker, 1909; Miura, 1928) and may rise during pregnancy to 0.9 per cent. (Harada, 1916). Such degrees of acidity must be of considerable importance in suppressing the growth of the great majority of organisms (Demme, 1927) and it would be expected that only those organisms which are highly resistant to acid media, e.g. *B. acidophilus*, yeasts and diphtheroids, would be found in the majority of cases of pregnancy. Recent work has shown that the vaginal bacillus of Döderlein is a member of the acidophilus group of bacilli.

The presence of such large amounts of lactic acid in the secretion of the vagina necessarily postulates a fermentable carbohydrate as a precursor, and the presence of glycogen in the vaginal wall led some workers to suggest that glycogen is the substance concerned (Zweifel, 1908; Döderlein, 1892; Krönig and Menge, 1897). In experimental work glycogen is not fermented by cultures of the vaginal bacillus (Miura, 1928), and it has also been observed that lactic acid may be present in the vagina of the newly born child before organisms have entered (Kienlin, 1926). It is, therefore, probable that the lactic acid in the vagina is not due to the direct action of the vaginal bacillus upon the glycogen in the vaginal wall.

In a recent comprehensive study Miura (1928) shows that a direct association exists between the activity of the ovaries, the deposition of glycogen in the vaginal wall, the presence of Döderlein's bacillus, and of lactic acid in the vaginal secretion. He has established that the same relationships hold for the higher monkeys but not for other animals, and has made experimental observations with regard to the influence of the ovaries on the vaginal secretion. Observations on the human subject following ovariectomy have also been made which corroborate the experimental work. According to Miura the amount of lactic acid produced in the human vagina in 24 hours varies from 15 mgm. to 8 mgm. (average value 12.17 mgm.), and the hydrogen-ion concentration runs parallel with the amount of lactic acid. Glycogen is laid down in the superficial layers of epithelium of the vagina only when the ovaries are present and are actively functioning. Extirpation of the ovaries or cessation of function, e.g. at the menopause, results in absence of glycogen, an alkaline reaction of the vaginal secretion, and a heterogeneous bacterial flora, with absence of Döderlein's bacillus. Extirpation of the uterus does not affect the character of the vaginal fluid. In cats, rats, dogs, rabbits and guinea-pigs, glycogen is not present in the vaginal wall, the vaginal secretions are alkaline, and Döderlein's bacillus is absent. Bremicker (1927) also has found that the vaginal fluid of animals with the exception of monkeys is alkaline and that glycogen is absent. The presence of glycogen in the vaginal wall in the embryo (Gragert, 1927) and in new-born children has been established by several observers (Kienlin, 1926; Kessler and Uhr, 1927; v. Jaschke, 1925) associated with acidity and Döderlein's bacillus. According to Menge the acid reaction persists for 3 to 4 weeks

after birth, when the vaginal secretion becomes much reduced in amount and alkaline in reaction.

Our knowledge of the characters of the vaginal fluid and of the vaginal flora of children from birth to adult life is not well established. The scattered observations of relatively few cases reported by the early workers served to indicate that the vaginal secretion in young girls is scanty in amount, alkaline, and free from Döderlein's bacillus. The more recent work is of conflicting nature. Kessler and Röhrs (1927) report that in 42 children from 1 to 8 days old vaginal bacilli were present in all cases (with streptococci in nearly all, staphylococci in many, and *B. coli* in a few cases) and that the pH varied from 4·0 to 6·0. In 38 girls aged from 9 days to 13 years vaginal bacilli were found in all cases together with other organisms. They emphasize the need of examining healthy children, because the laying down of glycogen in the vaginal wall is readily upset by constitutional illnesses, e.g. tuberculosis, as shown by Wölfring (1922). Thomas (1928), in the examination of children between 6 and 12 years of age, found Döderlein's bacillus only 20 times in 107 smears and isolated the organism in 8 cases. He found very few organisms of any kind in smears or cultures. From his description of the characters of Döderlein's bacillus other bacilli may have been included. Miura (1928), on the other hand, reports that the vaginal secretion of young girls is alkaline, contains few organisms of any kind, and that Döderlein's bacillus is absent until about the twelfth year when a change occurs associated with the onset of puberty; the secretion then becomes acid and Döderlein's bacillus becomes established. Soeken (1926) also reports a series of observations. In the course of weekly examinations of a girl for the detection of gonococci she found that a sudden change occurred from a coccal flora to a bacillary flora just before the onset of the first menstrual flow. Five hundred children of ages varying from 6 months to 9 years were then examined by means of direct films of the secretions. In no case was the picture suggestive of the flora of adult women. In 43 per cent. there were sparse cocci with individual bacilli, in 38 per cent. a moderate number of cocci with a few slender bacilli, and in 19 per cent. (mostly children under 2 years) a rich coccal flora with slender rods in moderate numbers. In a further series of 73 girls, 10 to 15 years old, a coccal flora was found in 39 cases and a bacillary flora in 34 cases. In the latter group puberty changes were present, whereas in the former group only 7 girls showed signs of puberty. As a result of weekly examinations over long periods Soeken found that the change from the coccal to the bacillary flora of the adult took place within a week and preceded the onset of menstruation. She expressed the opinion that the finding of the typical Gram-positive bacillary flora in the course of regular examinations is the first definite sign of the onset of puberty and more reliable than changes in the breasts or the development of hair. In the experience of the writer, children and young girls in hospital do not show the bacterial picture which is found in adults. Vaginal swabs in

young females are difficult to obtain free from contamination, and hospital patients, even though convalescent, may not be suitable material upon which to judge the normal, particularly if the deposition of glycogen in the vaginal walls is easily upset by local or constitutional disorders. The examination of a large series of adult non-pregnant healthy women, married or unmarried, obviously presents difficulty, and there is need for more information on the flora of such cases in order to establish the continuity of the bacterial pictures from birth to puberty and from puberty to the menopause and old age. If the association between ovarian activity, deposition of glycogen in the vaginal walls, and acidity of the vaginal secretion could be established it would reconcile many apparently conflicting results in this field of bacteriological work.

In the application to clinical work of the bacteriological findings in cases of pregnancy it has been customary to divide series of cases into groups corresponding to the 'grade of cleanliness' as it has been called (Schröder, 1921). Three groups are usually distinguished. In the first group (Grade 1) the vaginal secretion is tough, milk white, highly acid (pH 4·3 to 4·9), and contains a few epithelial cells and very numerous Döderlein's bacilli. In the second group (Grade 2) the secretion is thinner in consistency, the colour is yellowish or greenish white, the pH is 4·6 to 5·0, some leucocytes are present, and diphtheroids and cocci are present in considerable numbers with few Döderlein's bacilli. In Grade 3 the reaction is 5·2 to 6·8, plates of epithelium and many leucocytes are present, Döderlein's bacillus is absent, streptococci, coliform organisms, staphylococci, diphtheroids, yeasts and sarcinae are present, with *Trichomonas vaginalis* occasionally.

The figures obtained by various workers depend to some extent on the technique employed in taking the vaginal swabs, and special apparatus has been devised by some workers (Krönig, 1894; Lockhart, 1925). Contamination of the swab, however slight, by contact with the hymeneal ring may, for example, easily convert a Grade 1 preparation into a Grade 2. In pathological conditions of the cervix and vagina Grade 3 is frequent. There are, however, Grade 3 cases in which no obvious local pathological changes can be detected. If Miura's results are accepted it is possible that the determining factor in some Grade 3 cases is partial failure of ovarian function with absence of glycogen in the vaginal walls. Miura's work may also have some bearing on the ætiology of cases of leucorrhœa in which no obvious local cause can be found.

The relationship between the grades of cleanliness and the puerperal morbidity rate has been examined by a number of workers (Wirz, 1926; Neumann, 1925; Houlton, 1924; Bryce, 1928; Harris and Brown, 1928, &c.), but convincing evidence has not been obtained that the condition of the vagina *ante partum* affects the incidence of subsequent severe infections.

The changes which occur in the vaginal secretion during and after menstruation have been studied by Demme and Baltzer (1927) and by

Miura. During the menstrual flow the washing with blood, cervical mucus, and tissue fluids alters the growth relationships of the bacteria of the vagina, but Döderlein's bacillus is rapidly re-established as soon as the flow ceases.

The source of origin of Döderlein's bacillus in the new-born child is discussed by Thomas, who is of opinion that as the organism is associated with *B. coli*, staphylococci and diphtheroids it has origin from the faeces. Schweitzer (1919) has simultaneously examined the mouth, vulva, vagina and rectum of new-born children and reports the finding of Döderlein's bacillus in the mouth on the first day in 46 per cent. of cases and in the mouth, vulva, vagina and rectum in all cases on the third day. He supports the view which Krönig originally put forward that the infection of the child occurs from the vagina of the mother.

Extensive studies of the secretion of the uterus and vagina from the chemical and bacteriological aspects have been made by Schröder, Hinrichs and Kessler (1926). They also give detailed descriptions of the morphology and cultural characters of Döderlein's bacillus, and describe marked changes in morphology according to the pH value of the medium on which it is grown.

The identity of Döderlein's bacillus with *B. acidophilus* is asserted by Jötten (1922) on the basis of morphological, serological and cultural studies of numerous strains. Subsequent workers have accepted Jötten's findings. The resistance of the bacillus to highly acid conditions of culture is considerable (Demme, 1927; Miura, 1928). The strains of bacilli which Miura isolated from monkeys were indistinguishable from human strains. The organism can be cultured without difficulty from Grade 1 cases by stroking plates of hormone-agar, lactose-agar, or blood-agar and incubating under anaerobic conditions. Once isolated the organisms grow very well under aerobic conditions (see p. 376).

The numerous studies of the vaginal flora have thrown little light upon the ætiology of severe puerperal sepsis. The literature on this subject is very large and conflicting, mainly owing to the difficulties of proving the pathogenicity of strains of streptococci which have been isolated from the vulva or vagina (cf. Vol. II, p. 98).

A comprehensive survey of the literature on the subject of aerobic and anaerobic streptococci in the genital tract is given by Schäfer (1917). As a result of his study and of his own investigations he concludes that 'the organisms of the basic flora of the vagina are all non-pathogenic, atoxic, in human cases acidophilic, obligate and facultative anaerobic saprophytes, that obligate aerobic organisms seldom occur, that pyogenic bacteria occur very rarely and only as a passing phase'.

REFERENCES.

- BECKER, G., 1909, *Z. Geburtsh. Gynäk.*, **64**, 326.
BREMICKER, W., 1927, *Arch. Gynaek.*, **129**, 807.
BRYCE, L. M., 1928, *Med. J. Australia*, **2**, 102.
CAHANESCO, 1901, *Ann. Inst. Pasteur*, **15**, 842.

- CRUICKSHANK, R. & BAIRD, D., 1930, *Edin. Med. J.*, Sept., 135.
 DEMME, R., 1927, *Arch. Gynaek.*, **129**, 913.
 DEMME, R. & BALTZER, U., 1927, *Arch. Gynaek.*, **129**, 900.
 DÖDERLEIN, A., 1892, *Das Scheidensekret und seine Bedeutung für das Puerperalfieber*, Leipzig (O. Durr).
 GOENNER, A., 1887, *Zbl. Gynäk.*, **11**, 444.
 GRAGERT, O., 1926, *Arch. Gynaek.*, **128**, 43; 1927, *Zbl. allg. Path. path. Anat.*, **39**, 501.
 HAAG, 1915, cited from *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth.
 HARADA, T., 1916, *Amer. J. Med. Sci.*, **152**, 243.
 HARRIS, J. W. & BROWN, J. H., 1928, *Johns Hopk. Hosp. Bull.*, **43**, 190.
 HOULTON, C. L., 1924, *J. Obst. Gyn.*, **31**, 258.
 VON JASCHKE, R. T., 1925, *Arch. Gynaek.*, **125**, 224.
 JÖTTEN, K. W., 1922, *Arch. Hyg.*, Berl., **91**, 143.
 KESSLER, R. & RÖHRS, H. D., 1927, *Arch. Gynaek.*, **129**, 856.
 KESSLER, R. & UHR, E., 1927, *Arch. Gynaek.*, **129**, 844.
 KIENLIN, H., 1926, *Zbl. Gynäk.*, **50**, 644.
 KRÖNIG, B., 1894, *Zbl. Gynäk.*, **18**, 3 and 662.
 KRÖNIG, B. & MENGE, C., 1897, *Bakteriologie des weiblichen Genitalkanals*, Leipzig.
 LOCKHART, L. P., 1925, *J. Obst. Gyn.*, **32**, 49.
 MIURA, H., 1928, *Kyoto-Ikadangaku-Zasshi* (*Mitt. med. Akad. Kioto*), **2**, Heft 1.
 NEUMANN, H. O., 1925, *Z. Geburtsh. Gynäk.*, **89**, 303.
 SCHÄFER, P., 1917, *Arch. Gynaek.*, **106**, 407.
 SCHRÖDER, R., 1921, *Zbl. Gynäk.*, **45**, 1350.
 SCHRÖDER, R., HINRICHS, R. & KESSLER, R., 1926, *Arch. Gynaek.*, **128**, 94.
 SCHWEITZER, B., 1913, *Beitrag zur Bakteriologie der Scheide Schwangerer*, Leipzig, 1919, *Zbl. Gynäk.*, **43**, 641.
 SOEKEN, G., 1926, *Z. Kinderhik.*, **40**, 727.
 THOMAS, S., 1928, *J. Infect. Dis.*, **43**, 218.
 WIRZ, P., 1926, *Zbl. Gynäk.*, **50**, 2939.
 WÖLFRING, O., 1922, *Zbl. Gynäk.*, **46**, 1086.
 ZWEIFEL, 1908, *Arch. Gynaek.*, **86**, 564.

The Aciduric Group of Intestinal Organisms.

In 1899, Tissier isolated from the stools of breast-fed infants, by anaerobic methods, a Gram-positive non-motile non-sporing bacillus which he named *B. bifidus communis* because of its tendency to form bifurcations at one or both ends. He described the organism as a slender, slightly curved bacillus, pleomorphic, a strict anaerobe, forming small ovoid or lenticular colonies in sugar-agar, producing acid but no clot in milk and fermenting certain of the carbohydrates without gas-formation. In the following year, Moro reported the isolation from infants' faeces of a somewhat similar organism which was a facultative anaerobe and formed characteristic feathery colonies in deep beer-wort-agar. From its supposed enrichment in acid medium, Moro called the organism *B. acidophilus*, but Mereshkowsky and later Distaso showed that it is only acid-tolerant, not acidophile. Kendall has suggested the term 'aciduric' for these intestinal organisms which under cultural conditions survive a concentration of acid lethal for most other bacteria, but which do not find such a degree of acidity the optimum for their growth. To this group belong *B. bifidus* and *B. acidophilus* and numerous other organisms of similar type, which because of some particular morphological or cultural character have been given specific names. These acid-resistant intestinal organisms have been included in the class of lacto-bacilli: Gram-positive, non-motile,

non-sporing bacilli which have as their chief characteristic the formation of lactic acid and which are found mostly in dairy produce (milk, butter, cheese). Orla-Jensen (1919), however, separates the intestinal group from the true lactic-acid bacilli because of the ability of the former group to reduce nitrates, form catalase and show surface growth. A further distinction is their habituation to the intestinal canal: the true lactic-acid bacilli cannot become implanted in the intestine, as the experiments of Herter with *B. bulgaricus* conclusively proved. The aciduric group of intestinal organisms is apparently entirely saprophytic and is non-pathogenic to laboratory animals; a member of the group, however, *B. acidophilus odontolyticus*, has been regarded as the causal agent in dental caries (McIntosh, James and Lazarus-Barlow, 1922). In this group of acid-resistant organisms must be included Döderlein's vaginal bacillus, the predominant organism in the vagina after puberty and often present in practically pure culture during pregnancy; it has the morphological and cultural characters of the group and is possessed of the same acid-resistant properties as *B. bifidus* and *B. acidophilus*.

B. BIFIDUS COMMUNIS TISSIER.

A historical review of the literature on *B. bifidus* is given elsewhere (Cruickshank, 1925).

Morphology. A film made from the faeces of a breast-fed infant within the first month of life and stained by Gram's method shows an apparently pure culture of rather slender, straight or slightly curved Gram-positive bacilli of variable length—from 2 to 7 μ , although the majority are 4 to 5 μ , and 0.4 to 0.6 μ in breadth. The extremities are rounded and often at one or both ends there is a bulbous conical thickening. True bifurcation is rare, but small bacillary or coccoid offshoots, forming either an acute or obtuse angle with the main rod, are frequently seen, and perhaps more commonly a semblance of branching is given by the apposition of two or three bacilli forming a Y or triad. Diplo-bacillary forms are frequent, the two bacilli often forming an obtuse angle, the 'formes génicules' of Tissier, and two, three or four organisms may be seen lying closely apposed to each other—parallelism or palisade-formation. Many of the organisms in the smear from faeces retain the stain unevenly so that a granular or beaded appearance is produced. A film from a 24-hours' culture on agar shows the same slender rather pleomorphic Gram-positive bacillus, which morphologically most closely resembles the corynebacteria. An irregular thickening of one extremity or the grouping together of two or three organisms may occasionally produce a bifid appearance of the organism. *B. bifidus* loses Gram's stain rather readily, and in old cultures many Gram-negative forms are present. In older cultures pleomorphism is more in evidence; long curved threads staining unevenly appear in whorls and present a streptothrix-like appearance, but true branching has not been observed. *B. bifidus* is a non-sporulating, non-motile and uncapsulated organism.

Cultural characters. *B. bifidus* has been variously described as (a) a strict anaerobe (Tissier, 1899; Rodella, 1901; Cahn, 1901), (b) an organism requiring anaerobic or semi-anaerobic conditions for its primary isolation but which may become acclimatized to aerobiosis (Passini, 1903; Basten, 1914; Torrey, 1919; Cruickshank, 1925), and (c) a 'pleobiotic' organism which from being a strict anaerobe can be modified by selective cultivation into an aerobic spore-bearing phase and back again to its original anaerobic state (Noguchi, 1910). The difficulty of cultivation suggests that for primary isolation *B. bifidus* requires fairly strict anaerobic conditions, but after one or more subcultures the organism may be cultivated in the presence of air, particularly on media containing lactose or glucose. Surface colonies on such media are small, round and flat of 1 to 2 mm. diameter. They are greyish glistening discrete colonies which under the low power of the microscope appear delicately granular with a brownish centre and a finely serrated margin. After frequent subculture the growth is heavier and more confluent. In shake tubes of glucose- or lactose-agar, the colonies are dark brown ovoid or lenticular in shape and 1 to 2 mm. in diameter after 48 hours' incubation. The margin of the colony is at first regular and well-defined, but after 4 to 5 days' growth a lateral bud or offshoot frequently appears. The organism grows on media over a fairly wide range of reaction—pH 7·2 to 7·6 is probably the optimum—and because of its acid-resistant property it survives in fluid media of pH 3·5 to 4·0. The optimum temperature for growth is 37° C., but the organism grows at 22° C. and more slowly and less surely at room temperature. In fluid media *B. bifidus* produces a granular turbidity after 24 hours' incubation. Growth is rapid in glucose broth, and after 3 to 4 days' incubation the organisms form an abundant granular deposit leaving the supernatant fluid clear.

Primary isolation. Despite the enormous numbers present in the faeces of breast-fed infants, much difficulty has been experienced in the isolation of *B. bifidus* and many methods of isolation have been described. The following method has given satisfactory results: A deep tube of 1 per cent. lactose broth, pH 6·8 to 7·0, to which may be added a small piece of fresh sterile rabbit kidney, sealed with a half-inch layer of sterile vaseline, is inoculated with 0·5 c.cm. of a dense faecal emulsion: the vaseline is melted to seal the track of the pipette and the tube is incubated for 6 to 8 days at 37° C. Gas is evolved during the first few days and may be expelled by remelting the vaseline. Plates of 1 per cent. lactose-agar or of Loeffler's serum are then stroked from the primary culture and incubated anaerobically in a McIntosh and Fildes jar for 48 hours. Subcultures are made from the plates on glucose-agar slopes, into glucose broth or milk, and these are incubated aerobically. Rich inoculation is advisable since subculture from a single colony frequently fails to grow. After one or more subcultures on glucose-agar at two-day intervals the organism grows on ordinary agar and its viability becomes increased so that cultures on agar remain alive for several weeks at room temperature.

The addition of serum (5 to 10 per cent.) to solid media enriches the growth so that colonies in the depth of the medium become larger and appear as small whitish balls.

Biochemical reactions. *B. bifidus* is almost wholly saccharolytic in its activities. Indole is not formed and gelatin is not liquefied. Certain amino-acids and peptones are inimical to the growth of the organism, whereas the addition of 0.5 per cent. sodium caseinate to the medium seems to favour growth (Adams, 1925). There is no uniformity in the fermentation of sugars by different strains. Lactose and galactose are more readily utilized than glucose and saccharose (Kendall and Haner, 1924): glucose, lactose, maltose and saccharose are usually fermented, without gas-formation. Litmus milk is clotted in 48 hours by certain strains with decolorization commencing from the bottom of the tube. Growth on chocolate-agar results in a greenish halo indicating the production of hydrogen peroxide and the consequent oxidation of the blood-pigment (Macleod and Gordon, 1922).

Serology. Attempts to classify the different strains of *B. bifidus* serologically are no more successful than the attempted grouping according to their fermentation reactions. The results of agglutination reactions indicate that the different strains of *B. bifidus* are not serologically uniform (Cruickshank, 1925).

Distribution. The ubiquity of the aciduric group of intestinal organisms in the animal world was demonstrated by Mereshkowsky and his co-workers. They isolated an organism from the fæces of a series of animals ranging from molluscs to man, which they named *B. acidophilus* No. 1 and which morphologically resembled *B. bifidus* but was neither anaerobic nor bifid. Aerobic and anaerobic strains of the organism may be readily recovered from the fæces of mice, rats and rabbits (Webster, 1923; Cruickshank, 1925). *B. bifidus* is non-pathogenic to laboratory animals.

B. ACIDOPHILUS MORO.

B. acidophilus was first isolated by Moro (1900) and described as a Gram-positive bacillus, a facultative anaerobe, which tended to grow out into threads and which formed characteristic feathery colonies in deep beer-wort-agar. Finkelstein (1900) independently recovered a similar organism from infants' fæces by the use of acid glucose broth. In 1905, Grigoroff, and following him, Cohendy (1906), described an organism isolated from milk and apparently identical with *B. acidophilus*. Later workers (Rodella, 1901; Heinemann and Hefferan, 1909) concluded that *B. acidophilus*, the Boas-Oppler bacillus and the milk bacillus named *B. bulgaricus* were one and the same organism. It has since been shown that *B. bulgaricus* is not a normal inhabitant of the intestinal tract—its natural habitat is milk—nor can it become implanted in the gut (Herter and Kendall, 1909–10), although the organism was extensively used by Metchnikoff and his followers in their sour-milk therapy for intestinal toxæmia. *B. bulgaricus* also differs from *B. acidophilus* in certain cultural

characters, for example in its failure to ferment maltose (Rahe, 1914) or to grow under conditions of reduced surface tension (Albus and Holm, 1925). Recent studies have established the fact that the term '*B. acidophilus*' includes a group of organisms with certain characters in common but with differences in the individual strains as regards morphology, colony formation and sugar reactions (Rahe, 1914; Torrey, 1919; Cruickshank and Berry, 1924).

Morphology. Moro originally described *B. acidophilus* as a moderately thick, rather long Gram-positive bacillus with a tendency to curl or grow out into threads. Considerable pleomorphism appears, however, to exist among the various strains. Most of them are short slender Gram-positive bacilli (1 to 4μ by 0.4 to 0.7μ), occurring singly, in pairs, in short or long chains, or in palisade formation. Other strains are short thick coccobacillary forms while some organisms are curved or completely curled up. Even in pure cultures of a strain there may be great variation both in length and thickness. The pleomorphism is most marked in old cultures where long thread-like forms appear, beading or bipolar staining is common, and many of the organisms are Gram-negative. *B. acidophilus* is non-motile, non-sporing and uncapsulated.

Cultural characters. The optimum temperature for growth of *B. acidophilus* is $37^{\circ}\text{C}.$, and a slightly alkaline medium seems to be most suitable for the maintenance of the organism (Heinemann and Hefferan, 1909; McIntosh *et al.*, 1925). It is a facultative anaerobe and grows readily on ordinary agar. The addition of glucose or lactose enriches the growth. At first the colonies are discrete and the growth delicate, but after repeated subculture growth becomes more confluent. There are considerable differences in the types of surface colonies, which may increase in size to 3 or 4 mm. in 48 to 72 hours. Some are small and granular with raised brownish centre and irregular margin, others are flat, delicate and show feathery or filamentous processes. Very small round colonies with a ground-glass appearance, colonies in the form of a rosette, yellowish, slightly feathery colonies, and others opaque and striated have all been observed. There may be marked differences in the colonies of morphologically identical organisms. The classical feathery colony with its multiple hairy prolongations is less often seen in the depth of solid media than are the more compact 'crab-like' and lenticular colonies. Many strains of *B. acidophilus* do not grow readily in nutrient broth but all grow well in glucose broth.

Primary isolation. Many selective media have been described for the isolation of *B. acidophilus*, the basis of which is a reaction sufficiently acid to inhibit the growth of other organisms. For primary isolation a fluid medium should be used since in primary culture on solid media, *B. acidophilus* grows only when the organism is abundantly present in the fæces, although it may be cultured from the dejecta of some animals by direct plating on suitable solid media. Primary inoculation of the fæces into glucose or lactose broth containing 5 to 10 per cent. of N/1 acetic

acid is followed by subculture after 24 to 48 hours on such media as whey-agar (Rettger and Cheplin, 1921), casein-agar (Cannon *et al.*, 1920), liver glucose-agar (Torrey, 1917), or 2 to 4 per cent. lactose-agar. Cruickshank and Berry have recently described a method which obviates the use of special media and gives good results. To tubes of bouillon, adjusted to pH 7·6, glacial acetic acid is added in the proportion of 1 per cent. A thick emulsion of the stool is made in salt solution and 0·2 c.cm. added to 5 c.cm. of this broth. After incubation for 36 to 48 hours, the tubes are shaken up and a large loopful sown on plates of ordinary agar. These are incubated for 36 to 48 hours, and with few exceptions growth of acid-resistant organisms in pure culture is obtained. It is uncertain whether *B. acidophilus* actually multiplies in the acid fluid medium or merely survives a degree of acidity which is lethal to other organisms.

The isolation of *B. acidophilus* from the fæces of animals such as rat, rabbit, calf, is much more easily effected than from human dejecta. The viability of *B. acidophilus* varies with different strains: subcultures at weekly or more frequent intervals are necessary for some time after primary isolation.

Biochemical reactions. *B. acidophilus*, like *B. bifidus*, is almost wholly saccharolytic. There is great variability in the fermentation of sugars. Most strains ferment glucose and maltose without gas-formation and may in addition ferment lactose, saccharose and mannitol. Rahe (1918) suggested a division into four groups according to the action of his strains on glucose, maltose, lactose, saccharose and raffinose. Cannon, in the examination of 67 strains, agreed with Rahe's classification. Other workers have found it impossible to group the various strains according to fermentation reactions. Acid and clot is produced in litmus milk by most strains in 48 hours with decolorization from the bottom of the tube. The clot is soft and easily broken up and the whey is not expressed. Indole is not formed. There is no proteolytic activity: nitrates are reduced to nitrites.

Serological classification. Attempts to classify the aciduric group of organisms serologically have resulted in contradictory reports. Distaso (1911) stated that an antiserum to *B. bifidus* was without effect on *B. acidophilus*. McIntosh and his co-workers attempted to correlate different strains of *B. acidophilus* isolated from the fæces of adults and children, from stomach contents, from the fæces of monkeys and rats and from milk with those isolated from saliva and carious teeth, and concluded that 'members of the *B. acidophilus* group isolated from various sources have a moderately close relationship, those isolated from milk and human sources bearing a closer relation to *B. acidophilus odontolyticus* than those isolated from the intestinal tract of monkeys or rats'. Cruickshank and Berry failed to find any group relationship among their different strains, and only rarely was an organism agglutinable by a heterologous antiserum prepared against a morphologically similar organism. Jøtten claimed that *B. acidophilus*, *B. bifidus*, and Döderlein's bacillus are

serologically uniform, but Lash and Kaplan (1926) found that *B. acidophilus* was not agglutinated by an antiserum to Döderlein's bacillus. Investigation by the writers has suggested that some serological relationship exists between *B. acidophilus* and *B. bifidus*; for example a strain of *B. acidophilus* was agglutinated to titre by an antiserum to *B. bifidus*.

Distribution. The investigation of Mereshkowsky and his co-workers has shown that *B. acidophilus* is widely distributed as an intestinal organism among animals. *B. acidophilus* has also been isolated from milk, from saliva and from carious teeth, but not from soil or water (McIntosh and Lazarus-Barlow, 1924). Because of its pleomorphism numerous varieties have been described. A gas-producing strain was isolated by Torrey and Rahe (1915) from the faeces of 7 adults and 2 children in an examination of 37 individuals, and was also recovered from the dejecta of sheep and hens, but not from dogs, monkeys, rats, rabbits or guinea-pigs.

Other organisms of the same group have been described, e.g. *B. exilis* (Tissier, 1900), *B. intestinalis tuberculiformis* (Jacobson, 1908), *B. infantilis* (Herter, 1907), but these organisms appear to be variants of *B. bifidus* or *B. acidophilus*.

DÖDERLEIN'S BACILLUS.

In 1892, Döderlein reported the occurrence in the vagina of healthy pregnant women and virgins of a long Gram-positive non-motile bacillus which grew aerobically or anaerobically on glucose-agar as translucent 'dew-drop' colonies. He regarded this organism, which was subsequently named Döderlein's bacillus or *B. vaginae*, as the cause of the acid reaction in the vagina by the formation of lactic acid. Its morphological resemblance to the lactic acid bacilli and its apparent tolerance of an acid environment led Kruse and others (Schweitzer, 1913; Lehmann and Neumann, 1912; Naujoks, 1921) to classify the vaginal bacillus with the group of Gram-positive aciduric bacilli present in the intestine, and Jötten (1922) has pointed out the close similarity, morphologically, culturally and serologically, between Döderlein's bacillus and *B. acidophilus*.

Morphology. A direct smear taken from the vagina during the later months of pregnancy and stained by Gram's method shows in a large proportion of healthy women an almost pure growth of a slender Gram-positive bacillus, straight or slightly curved, occurring singly, in pairs, or in small bundles. Shorter stouter forms may be seen, but it is difficult to decide whether these represent true Döderlein's bacilli or are diphtheroid organisms which are not uncommonly present in the vaginal flora. Films from culture show the same type of organism, which in its morphology resembles most closely the aciduric group of intestinal organisms. Like *B. acidophilus*, different strains of Döderlein's bacillus show considerable pleomorphism in cultures: there are short and long, thick and thin forms, occurring singly or in pairs or parallel rows, chains of bacilli or coccobacilli, and, particularly in older cultures, long thread-like forms, Gram-negative bacilli and involution forms (Jötten, 1922). Chain-formation

is a feature of some strains, and a film made from a culture of such a strain shows a 'whorled' and tangled arrangement. In films made from a culture in fluid media, the 'beading' or granule staining may closely resemble that of the diphtheroid group. A Pugh- or Neisser-stained film does not as a rule show the presence of chromatic granules. The bacillus is non-motile, non-sporing and uncapsulated.

Cultural characters. Although anaerobic conditions are most favourable for the primary isolation of Döderlein's bacillus, the organism once isolated grows readily in the presence of oxygen (cf. *B. bifidus*). On the surface of hormone-agar or of lactose-agar, very small, translucent, round and slightly raised discrete colonies appear after 24 to 48 hours' incubation. There is a pleomorphism in the colony-formation as in the organism itself. While some colonies are round and compact with a brownish centre, others are more delicate with filamentous offshoots. A detailed description of the structure of the colonies is given by Jötten (1922). In broth there is at first a uniform turbidity followed later by a rather compact granular sediment and a clear supernatant fluid. The optimum growth is obtained with a medium which is slightly alkaline in reaction: strongly acid medium is inhibitory to the growth of Döderlein's bacillus (Rother, 1922).

Primary isolation. As with *B. acidophilus* and *B. bifidus*, some difficulty has been experienced in the cultivation of Döderlein's bacillus and consequently various methods have from time to time been advocated for its isolation. We have found that the organism may be readily recovered by the following method: the vaginal swabbing is inoculated primarily into 1 per cent. lactose broth which is incubated either aerobically or anaerobically for 24 to 48 hours. Subcultures are made on lactose-agar plates, on which, if the lactose broth has shown a good growth of Döderlein's bacillus, many colonies appear in 48 hours under aerobic or anaerobic conditions. If other organisms are present in the lactose broth, anaerobic incubation of the subculture on lactose-agar will favour the growth of the vaginal bacillus and inhibit the others. Primary inoculation on lactose-agar incubated either aerobically or anaerobically gives a good growth of the organism, particularly if a direct smear of the vaginal swabbing shows an abundant and apparently pure flora of Döderlein's bacillus. Serum-agar and blood-agar have also been found to be suitable media for the primary isolation of the organism. It may be maintained by subsequent culture, at first at frequent intervals, on hormone-agar. The addition of sugar to the medium favours the growth of the organism. Rother (1922) found that serum and ascitic fluid enhanced growth only so far as these fluids contained glycogen, and further, that glycogen could not be utilized by the organism until it was converted to sugar by enzyme action.

Döderlein's bacillus, when dried on silken threads, remains viable for 12 months. The organism is killed by heating at 60° C. for 20 minutes and at 70° C. for 5 minutes.

Biochemical reactions. The vaginal bacillus is a saccharolytic organism. It does not liquefy gelatin nor form indole. Nitrates are reduced to nitrites. It is a powerful producer of acid, forming as much as 150 c.cm. of normal acid per litre of glucose broth. Milk is clotted and decolorized in 2 to 3 days: there is no expression of the whey. The fermentation reactions with sugars are not sufficiently uniform to afford a basis for classification (Thomas, 1928). The organism resembles *B. acidophilus* in fermenting maltose.

Serology. Very little work has been done on the serological relationship of Döderlein's bacillus to other strains of the same species or to the group of aciduric organisms. Jötten (1922) found that spontaneous agglutination in saline or normal serum occurred so frequently as to preclude a classification based on the agglutinability of the various strains of the organism and of *B. acidophilus* by antisera prepared against one or more strains of each of these bacilli (Döderlein's bacillus and *B. acidophilus*). By the method of complement fixation, however, he was able to demonstrate a close serological relationship existing between *B. acidophilus* and the vaginal bacillus. There was no fixation of complement when emulsions of *B. coli*, *B. anthracis* and *B. subtilis* were used as control. Lash and Kaplan failed to demonstrate any cross-agglutination between strains of Döderlein's bacillus and the intestinal *B. acidophilus*, while Thomas remarks that 'there is no grouping of strains possible by the agglutination tests'.

Distribution. The organism has been described as occurring in the mouth and intestines of young children (see p. 336) as well as in the genital tract.

REFERENCES.

- ADAMS, A., 1925, *Jahrb. Kinderhkl.*, **110**, 186.
 ALBUS, W. R. & HOLM, G. E., 1925, *Proc. Soc. Exp. Biol.*, N.Y., **22**, 337.
 BASTEN, J., 1914, *Z. Hyg. InfektKr.*, **77**, 282.
 CAHN, 1901, *Zbl. Bakt.*, Abt. I, **30**, 721.
 CANNON, P. R., 1924, *J. Infect. Dis.*, **34**, 227.
 CANNON, P. R., DRAGSTEDT, L. R. & DRAGSTEDT, C. A., 1920, *J. Infect. Dis.*, **27**, 139.
 COHENDY, M., 1906, *C.R. Soc. Biol.*, Paris, **60**, 364.
 CRUICKSHANK, R., 1925, *J. Hyg.*, Camb., **24**, 241.
 CRUICKSHANK, J. & BERRY, D. W., 1924, *Brit. Med. J.*, ii, 944.
 DISTASO, A., 1911, *Zbl. Bakt.*, Abt. I, Orig., **59**, 48.
 DÖDERLEIN, A., 1892, *Das Scheidensekret und seine Bedeutung für das Puerperalfieber*, Leipzig (O. Durr).
 FINKELSTEIN, H., 1900, *Deuts. med. Wschr.*, **26**, 263.
 GRIGOROFF, S., 1905, *Rev. med. Suisse rom.*, **25**, 714.
 HEINEMANN, P. G. & HEFFERAN, M., 1909, *J. Infect. Dis.*, **6**, 304.
 HERTER, C. A., 1907, *Bacterial Infections of Digestive Tract*, New York.
 HERTER, C. H. & KENDALL, A. I., 1909-10, *J. Biol. Chem.*, **7**, 203.
 JACOBSON, G., 1908, *Ann. Inst. Pasteur*, **22**, 300.
 JÖTTEN, K. W., 1922, *Arch. Hyg.*, Berl., **91**, 143.
 KENDALL, A. I., 1908-9, *J. Biol. Chem.*, **5**, 419.
 KENDALL, A. I. & HANER, R. C., 1924, *J. Infect. Dis.*, **35**, 77.
 KRUSE, E., 1910, *Allgemeine Mikrobiologie*, Leipzig.
 LASH, A. F. & KAPLAN, B., 1926, *J. Infect. Dis.*, **38**, 333.
 LEHMANN, K. B. & NEUMANN, R. O., 1912, *Atlas und Grundriss der Bakteriologie*, Munich.

- MCINTOSH, J., JAMES, W. W. & LAZARUS-BARLOW, P., 1922, *Brit. J. Exp. Path.*, **3**, 138; 1924, *ibid.*, **5**, 175; 1925, *ibid.*, **6**, 260.
- MCLEOD, J. W. & GORDON, J., 1922, *J. Path. Bact.*, **25**, 139.
- MERESHKOWSKY, S. S., 1905, *Zbl. Bakt.*, Abt. I, Orig., **39**, 380.
- MORO, E., 1900, *Wien. klin. Wschr.*, **13**, 114.
- NAUJOKS, H., 1921, *Zbl. Bakt.*, Abt. I, Orig., **86**, 582.
- NOGUCHI, H., 1910, *J. Exp. Med.*, **12**, 182.
- ORLA-JENSEN, S., 1919, *Lactic Acid Bacilli*, Copenhagen.
- PASSINI, F., 1903, *Jahrb. Kinderhkl.*, **57**, 87.
- RAHE, A. H., 1914, *J. Infect. Dis.*, **15**, 141; 1918, *J. Bact.*, **3**, 407.
- RETTGER, L. F. & CHEPLIN, A. A., 1921, *The Transformation of the Intestinal Flora*, Newhaven and London.
- RODELLA, A., 1901, *Zbl. Bakt.*, Abt. I, Orig., **29**, 717.
- ROTHER, W., 1922, *Zbl. Bakt.*, Abt. I, Orig., **88**, 558.
- SCHWEITZER, B., 1913, *Zur Prophylaxe des Wochenbettfiebers*, Leipzig.
- THOMAS, S., 1928, *J. Infect. Dis.*, **43**, 218.
- TISSIER, H., 1899, *C.R. Soc. Biol.*, Paris, **51**, 943; 1900, *La Flore intestinale chez le Nourrisson*, Paris.
- TORREY, J. C., 1917, *J. Bact.*, **2**, 435; 1919, *J. Med. Res.*, **39**, 415.
- TORREY, J. C. & RAHE, A. H., 1915, *J. Infect. Dis.*, **17**, 437.
- WEBSTER, L. T., 1923, *J. Exp. Med.*, **37**, 21.

CHAPTER XII. SWINE ERYSIPELAS.

(French, *Rouget* ; German, *Rotlauf*.)

BY JAMES T. EDWARDS (LISTER INSTITUTE, LONDON).

Introduction.

SWINE erysipelas and swine fever are the most important infectious diseases of the domesticated pig. In certain parts of the world, as in the United States of America, swine erysipelas has been recorded only very rarely, as a benign infection, whereas the ravages of swine fever (hog cholera) have been so formidable that it has been the most pressing economic consideration of the pig breeder. In other parts, as on the Continent of Europe, on the other hand, swine erysipelas has prevailed, as a more or less serious infection, for at least a century, to a degree which has generally rivalled that of swine fever. In Great Britain it has usually been taught that swine erysipelas is a relatively mild disease, of sporadic occurrence ; its frequency and severity have not been subjected, however, to detailed study, largely for the reason that it has not been classed as a notifiable disease ; but it is known to occur in certain districts, as in the Eastern Counties, often as a very serious and somewhat widespread infection, generally of greater importance to the pig owner than is swine fever. Again, as on the Continent of Europe, the disease has a local and seasonal incidence, tending to become aggravated in certain years, when it may appear as an acute infection simultaneously in counties so widely separated as Norfolk and Cornwall.

Historically, swine erysipelas is of considerable interest to the bacteriologist and immunologist, as it was with this infection that Pasteur and Thuillier (1882, 1883) first demonstrated the method of artificial immunization by means of live cultures graded in virulence by animal passage ; passage through the rabbit, which is relatively resistant, was claimed to attenuate the virulence of the causal organism for the pig ; passage through the pigeon, which is much more susceptible, was held to exalt it. In active immunization, therefore, pigs were first injected with broth cultures of the rabbit virus, and, after an interval, with cultures of the pigeon virus. The method (except in Hungary) was never adopted, even in France, on a large scale, largely because accidents occurred with distressing frequency following upon the inoculations, and it has long been superseded entirely by preventive inoculation, in which antiserum, used alone or simultaneously with live culture, is employed. It is almost certain that the broth cultures of Pasteur and Thuillier were not pure cultures of the causal organism of swine erysipelas, and the first accurate description of its morphology and cultural characteristics is attributable to Loeffler (1885). Following upon the identification of the causal organism of the acute septicæmic form of infection, other forms of disease in the pig, such

as the peculiar urticarial condition (‘diamonds’, nettle rash, Jensen, 1891), the chronic verrucose endocarditis (Bang, 1892), and the occasional arthritic forms, were proved to be the localized manifestations of less acute infection with the same organism.

The Disease in the Pig.

The symptoms and lesions, which are described at length in veterinary text-books, vary considerably with the virulence of infection. (1) The acute, septicæmic form, with high fever, great prostration, and, later, diffuse red or livid patches on the body surface, with death within four days. Recovery is rare, and sometimes the progress of the disease may be so rapid that death occurs before reddening of the body surface takes place (the so-called *rouget blanc* of French authors). (2) The subacute, urticarial form (*Nesselfieber*, urticaria, ‘diamonds’) in which the symptoms of fever and malaise are much less distinct, and large, sharply circumscribed, hæmorrhagic patches, often quadrilateral in shape, appear on the skin; recovery is usual. (3) The chronic forms, which sometimes supervene on the acute forms, but usually, in England at least, appear without preceding acute infection. The symptoms are merely those of a more or less pronounced unthriftiness, and the common lesion is a massive fibrinous deposit (‘verrucose endocarditis’) on the mitral valve of the heart, rarely on the other valves; more seldom, arthritic troubles are seen. These clinical forms may merge imperceptibly into each other. In the septicæmic form there is seen intense congestion of the internal organs and subcutaneous tissue, with enlargement of the spleen and lymphatic glands.

The Bacillus of Swine Erysipelas.

Nomenclature.

The most recent attempt at specific classification, that of the Committee of the Society of American Bacteriologists (1920), has allotted to the bacillus the designation *Erysipelothrix rhusiopathiæ* (Family, Actinomycetaceæ; Order, Actinomycetales; Class, Schizomycetes). The specific name would appear to be derived from Kitt’s (1894) term, *rhusiopathia suis*, for the disease, and the generic term from Rosenbach’s (1909) view that the organisms of swine erysipelas, mouse septicæmia, and human erysipeloid deserved separate specific names; the observation of streptothrichal forms in certain culture media and chronic lesions would justify the introduction, it was believed, of the word. Otherwise, in veterinary writings, the organism has been commonly referred to by the term *Bacillus erysipelatis suis*, which is more descriptive perhaps than in strict accord with taxonomic rectitude. The mass of evidence now indicates that the organisms similar to that of swine erysipelas which naturally infect different host species, and, commonly, exhibit a striking and exclusive adaptation to the separate species are, in reality, antigenically the same and reveal no definite characteristics that would otherwise merit their separation into different species. Neither can the appearance of streptothrichal forms be regarded as a characteristic property shown by the

organisms, any more than it can when it is observed, occasionally, in such different bacterial forms as the Pasteurella and acid-fast groups. In some respects the organism of swine erysipelas shows some resemblance to the Corynebacteria (morphology, staining, absence of spore formation), one of which (*C. pyogenes*) is a common cause of suppuration in swine. It shows, however, certain aggregate peculiarities which do not accord with those of other groups, notably in regard to its natural history, pathogenic action and oxidation-reduction properties in artificial culture media, so that it may still be held to be *sui generis*.

Morphology and staining reactions.

Short, slender, straight or slightly curved rods; 1.0 to 1.5 μ long (Hutyra and Marek). This is the form seen usually in the fresh blood of naturally diseased (septicæmia) or experimentally infected animals. In broth cultures the organisms show a tendency to grow into long filaments, which may show thickening, and occasional branching; filamentous forms may also be seen in the chronic lesions (endocarditis). In blood smears the bacilli are found scattered, often in very large numbers, in the plasma, and sometimes in clumps; they are also abundant within the cytoplasm of the polynuclear leucocytes. In the chronic lesion, verrucose endocarditis, they are found closely packed as a fringe just within the free surface of the lesion, where they show up very prominently after Gram staining on examining a section under the low power of the microscope. The involution forms displayed prominently by the organism in cultures have been studied by Sabella (1925), employing media containing saponin; he concludes that the conditions which are conducive to the production of these forms are the same as those which obtain for other bacteria, namely those which promote slow, restricted growth of the organism, during which time it is adapting itself to the change in environment. The organism stains well by the ordinary basic aniline dyes and by Gram's method; it is one of the most Gram-fast of the pathogenic bacteria; it is not acid-fast; it is non-motile, and does not bear spores.

Cultural characteristics.

The organism grows readily, but meagrely, on most of the ordinary laboratory media, and growth, which proceeds at first quickly at body temperature, soon comes to an end. It is usually considered to be easily maintained in stock cultures.

The most characteristic growth is seen in stab gelatin. At ordinary room temperature, this begins with the appearance, in two to three days, of small, pinhead, white masses along the needle track, which later join up; a slight turbidity is then seen in the surrounding medium, which next gives place to fine threads radiating horizontally from the central core, which permeate the medium, often in tufts, in all directions. The growth proceeds almost uniformly along the whole length of the inoculated column, being restricted only towards the top a little, so that it presents typically

a bottle-brush appearance. With unsuitable medium, this appearance may fail not infrequently to develop. There is no liquefaction, but slight softening.

On agar slopes, minute dew-drop colonies appear within 24 hours' incubation at 37° C., which show little or no tendency to further development. They are not unlike in appearance the smooth colonies which often appear after sowing out on slopes morbid material containing virulent streptococci. With richer seed material, a thin glistening film is seen, which is usually not continuous, unless the seed is very rich, as the colonies show little tendency to fusion. The growth is emulsified readily in water. In agar plates, the colonies are transparent, tiny, convex or spherical structures, about 1/10 mm. in diameter.

In broth, a slight uniform turbidity appears during the first two or three days of incubation, which later settles as a small flocculent mass at the bottom of the tube. Meat broth containing 2 per cent. peptone adjusted to pH 7·8 is said to yield most abundant growth. The addition of a small quantity of sterile serum or of glucose has been claimed to improve growth, but the action of glucose is said to depress the virulence of cultures. The growth on solid serum is poor, and resembles that seen on agar slopes. Growth proceeds relatively well in fresh liquid serum, and also—a point of some importance—in a specifically immune antiserum. On potato slopes no macroscopically visible growth is seen, although the early observers reported that growth was obtainable when the medium was maintained *in vacuo*.

In view of its growth characteristics, it has been customary to describe the organism as a 'facultative aerobe' or 'microaerophilic'. Further work needs to be done, however, to specify more accurately its oxidation-reduction requirements, the cruder observation of which as they affect it in the common laboratory media having served usefully for long to distinguish it readily from other kinds of bacteria.

The reactions in special media used for the differentiation of other groups of bacteria have no considerable practical interest. It ferments no sugars, except sometimes dextrose and lactose, and produces no change, or only slight acidity in litmus milk. Cornell and Glover (1925) have stated that in testing the identity of the bacillus isolated by them from an outbreak of joint-ill in lambs with a strain of the swine erysipelas bacillus of porcine origin, both produced acid, but not gas, in lactose, glucose, maltose, lævulose; slight acid in inulin; no change in saccharose, mannitol, dulcitol, salicin, arabinose and glycerol; there was slight acidification of litmus milk, without clot. The bacillus of swine erysipelas does not form indole; forms hydrogen sulphide (readily appreciable when the organism is grown in massive broth cultures); gives catalase reaction; reduces nitrates slightly. Ordinarily, it shows little tendency to hæmolyse red blood corpuscles, but is said to produce hæmolysis around deep colonies in agar plates. The occurrence of 'rough' and 'smooth' (R and S) types of colonies (variants) has been reported by

Spryszak and Szymanowski (1929) : the rough variants were caused to appear by passaging the organisms through broth media containing 20 per cent. of immune serum.

Resistance.

The recorded evidence would show that the swine erysipelas bacillus is no more resistant to the effects of physical and chemical agents (heat, direct sunlight, desiccation and disinfectants) than are the generality of other non-spore-bearing bacteria ; but in the outside world it shows remarkable tenacity in certain circumstances. In broth cultures, for example, it is killed by heating to 55° C. for five minutes. On the other hand, it survives for longer than four months in putrefying meat, and can apparently survive for months in buried carcasses and pieces of flesh. It has been found alive after 170 days in pickled pork, 30 days in smoked pork, and 30 days in salted (common salt and saltpetre) pork ; it succumbed in small pieces of pickled pork subject to two successive smokings. It was found alive after 17 days in ordinary water and 34 days in distilled water. Such observations of prolonged survival of the organism in the outside world led certain of the early observers to conclude that it must develop some form of protective capsule, but there is no evidence whatever in reality of the possession of a resistant structure, and the phenomenon of survival is probably to be explained simply in the light of the oxidation-reduction requirements of the organism.

Habitat.

There is a good deal of evidence, mostly indirect, to indicate that the organism may lead a saprophytic existence. This is brought forth by a number of facts : The natural history of outbreaks, pointing to an origin in soil infection rather than in spread from other centres of infection ; the known prolonged survival in a state of high virulence in certain materials ; the discovery of the organism in the alimentary tract of pigs healthy in appearance (tonsils, gall-bladder, crypts of the ileo-cæcal valve, fæces) ; the independent infection of other species (mice, sheep, fowl) with otherwise indistinguishable organisms, naturally confined in pathogenic action to the separate species, suggesting adaptation to the species from a common saprophytic ancestor. In the diseased pig, the organism is frequently found in large numbers in the circulating blood, particularly in the leucocytes, but sometimes it may be so rare at the time of examination that resort must be had to cultural tests or animal inoculation. In the chronic forms of the disease, the organism is usually readily discoverable in the lesions, and a few may be recovered easily on cultivation from the circulating blood. A progressive form of infection, manifested by prostration after artificial inoculation, has been described in the white rat, in which often no organisms can be cultivated from it at the time of death.

Pathogenic action : mode of infection and method of spread.

Artificially, in the laboratory, the bacillus of swine erysipelas is rapidly fatal as a rule to mice (white and grey) and pigeons after inoculation, and these animals are the ones commonly used in diagnostic tests and tests upon the potency of antisera. A dose as low as 0·000001 c.cm. of a 24-hour broth culture commonly produces fatal infection in the mouse, after intraperitoneal injection, with death in four or five days ; larger doses may produce death within two days, from acute septicæmia. In serum potency tests, however, it is necessary to keep the mice under observation for a week or longer. The pigeon also succumbs to an acute septicæmia after inoculation into the breast muscles, but the dose required is larger than that for the mouse. The rabbit is also susceptible, to a much smaller degree, however, and inoculation of a large dose subcutaneously often produces only a local swelling, from which the animal recovers. The guinea-pig, fowl and field mouse are resistant.

Transmission of the disease to the pig usually succeeds by inoculation or scarification with material from the acute natural disease or recently isolated cultures of the organism, but even this drastic method often fails with material from chronic lesions or old cultures. Thus, for example, the British Departmental Committee on Swine Fever (1896, 1897) in their original examination of a number of chronic cases (endocarditis) in Great Britain failed to transmit infection to the pig by any channel. Again, although the acute natural disease spreads rapidly in the scene of outbreaks, attempts at transmission by feeding with morbid material or cultures usually fail. Nocard and Leclainche state, however, that the most certain method of transmitting the disease experimentally is by feeding with the viscera of an animal that has just succumbed to the disease. It has been objected by other workers that the possibility of introduction by abrasions is not excluded in this method. Huttyra and Marek conclude that natural infection usually takes place through the alimentary tract, and more seldom through skin lesions, but that the mechanism of entrance does not seem to be adequately explained hitherto. Virulent bacilli are excreted in large numbers in the fæces and urine of pigs suffering from the acute disease.

The spread of the disease is facilitated in wet years, and outbreaks are particularly frequent in low-lying districts ; they appear with increased severity during the warmer months of the year (May to September), when they may be seen to arise simultaneously in widely separated enzootic centres, and abate during the winter months. The chronic disease, as was observed by the British Committee, may appear sporadically among groups of pigs, and show little or no tendency to spread from the affected individuals ; in such cases, there is often no history of previous contagion.

Young pigs, under three months of age, show a high degree of resistance, but thereafter their susceptibility increases until they are 10 to 12 months old. Animals of the finer, improved breeds are said to be much more susceptible than are those of the coarser breeds. Wild pigs are resistant.

Cohen (1927), in an extensive epizootiological study of the disease from 1898 to 1924, found that the incidence of morbidity among swine throughout Germany varied annually from 0·42 to 0·99 per cent., the figures showing a considerable rise during the War years. The normal annual death-rate, among the total swine population, did not exceed 0·17 per cent. (though it rose during the War to 0·3 per cent.). The total number of swine that died or were slaughtered on account of the disease never exceeded 0·8 per cent. The largest number of cases occurred in the third quarter of the year. The estimated annual financial loss through death alone during the period amounted to over a million marks. Geographical position, numbers of piggeries in an area, trade and movement, and soil conditions were the most important factors influencing spread. Control of the disease cannot be accomplished by sanitary police measures alone ; it is necessary to resort also to preventive inoculation, the method of sero-vaccination, even though it may possibly spread the infective agent, being the most useful yet known to prevent increased losses.

Immunity.

The economic importance of the disease in the pig on the continent of Europe has attracted a great deal of attention to methods of artificial immunization, and since the early Pasteurian researches there has accumulated in the Continental literature a voluminous record of evidence upon this problem, and upon the probable mechanisms of immunity. No exotoxin has been demonstrated in cultures of the bacilli, and so the immunity conferred upon pigs cannot be held to be due to any antitoxic property of their body fluids. Moreover, immune animals do not manifest any immediate direct bactericidal action on the part of their fluids towards inoculated living bacilli : thus, Voges and Schütz (1898) showed that after inoculation with the second Pasteurian vaccine, and after simultaneous injection of antiserum and living virus (sero-vaccination), the blood of pigs was teeming with bacilli, which, nevertheless, caused no visible disturbance in the pigs ; Prettner (1906) found that passively immunized mice harboured virulent infection in their tissues for 24 hours to 3 days after inoculation of culture. The duration of active immunity following upon the inoculation of attenuated cultures, as in the Pasteurian method, or as employed in sero-vaccination, is not prolonged, not exceeding four to five months (Preis), although this would appear to depend to some extent upon the virulence of the inoculated bacilli. Inoculation with dead cultures (killed by heat) is not followed by immunity. Nevertheless, a very potent prophylactic serum can be obtained from animals by repeated inoculation with massive doses of the live bacilli. This was first prepared, in experimental studies, by Lorenz, in Baden, from immunized rabbits (1892) and later (1894) from pigs. Leclainche (1899), in France, was the first to record the preparation of the serum by the immunization of horses, which are insusceptible to infection. Later, it was shown that an equally efficacious serum can be prepared from other resistant large animals, such as cattle and buffaloes and dogs.

The preparation of antiserum is undertaken on a considerable scale in various serum institutes on the Continent, and in Germany its commercial issue has been subject for some time to certain official standardization tests. The mode of preparation varies a little in different institutes, but generally consists in the repeated intravenous inoculation of horses, at 8- to 10-day intervals, with living broth cultures. Massive inoculation results after a time in the appearance of serious local complications (joints, tendon sheaths, heart), but it does not seem necessary to press forward inoculation to this extent, and 100 to 140 litres of serum should be obtained from a horse in the course of a year. The horses react rather strongly after each inoculation of culture. In the German official tests the serum prepared is tested concurrently with a standard dried serum, which in a dose of 0.002 gm. protects a mouse against 0.01 c.cm. of a virulent 48-hour broth culture; this quantity of serum is said to contain two 'immune units'. The injections are made subcutaneously, that of the live culture twenty-four hours after that of the serum. The control mice should die within three days; the inoculated animals are kept under observation for eight days. The tests devised by Leclainche (France) comprise the use of the pigeon, inoculated with a mixture of the serum and live culture.

The immune serum has no direct action upon the bacilli, which remain alive and fully virulent after 24 hours' contact with it at 37° C. (see, however, earlier reference to production of 'rough' variants in media containing immune-serum, p. 383). There is no absorption of immune-bodies as the result of the direct contact. The protective action of the serum has been ascribed by some workers to an opsonic effect, but this would hardly explain the increase in numbers of organisms seen in immune animals following upon inoculation. The observed facts follow rather closely those studied in the immunity against fowl cholera and anthrax, and, as has been found with the latter infection, the reticulo-endothelial apparatus may be the mechanism that is affected mainly in the establishment of immunity. It has been claimed that an Ascoli (precipitin) reaction can be readily obtained with the use of immune-serum and extracts of infected tissue, but a reaction of the same kind is also appreciable with extracts of non-infected material.

In the prophylactic control of outbreaks in enzootic centres, the use of serum alone, for passive immunization, the effects of which last only a few days, is not recommended on the Continent. Instead, there is inoculated simultaneously with (or twenty-four hours after) the serum a dose of live culture (sero-vaccination) followed sometimes after an interval by a dose of live culture only. The system is practised on a very large scale and appears to yield very satisfactory results. It is very difficult, however, to obtain an estimate of its exact value among a large inoculated pig population (see earlier reference to Cohen's investigations in Germany, p. 385). In England, at the present time, in the scene of actual outbreaks, the serum is employed alone. The serum has also a considerable curative

action. The dose for prophylactic purposes in the pig generally varies from 5 to 15 c.cm.

Similar Infections in Species other than the Pig.

The existence of infections in certain other species, such as mice and human beings, bearing a close ætiological relationship with swine erysipelas has long been known, but of recent years there have been published accounts of disease, amounting sometimes to considerable outbreaks, in still more species of animals, so that although infection with this type of organism assumes greatest significance in the pig, it is by no means confined to this species. The more important infections thus described affecting other animals are the following :

Mouse septicæmia.

This infection was first described by Koch (1880) following upon the inoculation of mice with putrid blood. It was later discovered by Loëffler as a cause of natural outbreaks among mice. One of the most interesting accounts of the disease in the literature is that of the extensive outbreak which recently occurred among field-mice (native meadow mice, *Microtus californicus estuarensis*, and house-mice, *Mus musculus*) in California (Wayson, 1927) in circumstances in which there would seem to be no association with the natural porcine infection. It has been stated earlier that field-mice are resistant to infection with cultures of the pig bacillus. Moreover, with the widespread occurrence of the pig disease in Europe, one would have otherwise expected frequent contamination of mice. Earlier observers (Preisz) believed that the mouse bacillus showed some slight differences in cultural and other characters which entitled it to the designation *Bacillus murisepticus*. The differences have not been considered noteworthy by later workers. The bacillus shows the same range of pathogenicity for small laboratory animals as does the pig bacillus, but attempts to infect pigs with it have generally been unsuccessful. The identity of the organisms has been shown by various immunological tests. They behave in the same manner, in precipitin and agglutination tests, towards an immune serum.

Sheep disease.

An outbreak of polyarthritis caused by this organism in a flock of sheep was first described by Poels (1913), and his findings were later confirmed in similar outbreaks in Europe. Christiansen (1919) encountered a more severe type of infection, with high mortality, in a flock of young lambs, in which the morbid changes were hæmorrhagic enteritis, great enlargement of the mesenteric glands, degenerative changes of the organs, and hæmorrhages under the endocardium and epicardium. Cornell and Glover (1925), in their studies of outbreaks of polyarthritis in lambs in England, which they found to be mainly attributable to streptococcic infection, give a careful description of one serious outbreak in which the organism responsible was indistinguishable from that of swine erysipelas by morphological and

cultural examination, agglutination tests and passive immunization tests upon mice. The organism isolated proved rapidly fatal upon inoculation to lambs but not to the pig. Lambs proved susceptible to infection after massive feeding with cultures. These animals, however, were resistant to inoculation with a virulent strain of porcine origin. The same organism would seem to be responsible for polyarthritides in lambs in the United States of America (Hadleigh Marsh, 1929) and, commonly, in Australasia (Hopkirk and Gill, 1930; personal examination of cultures from Seddon). It is noteworthy that in these cases the sheep disease was not associated in any way with the prevalence of the pig disease.

Bird disease.

There are a number of scattered observations of infection with this organism in various species of birds. It was first described by Jarosch (1904) as a septicæmic condition in the turkey, and subsequently by others as the cause of outbreaks among fowls, ducks and pigeons, water-hen and parroquet. In the fowl disease the affection was of the acute septicæmic type, whereas that described by Pfaff (1921), at Prague, was a chronic wasting condition with diarrhoea, against which an anti-swine-erysipelas serum gave excellent results in preventive and curative treatment. The bird disease, however, is relatively uncommon.

Cattle disease.

In cattle, the infection has very rarely been discovered.

Human disease.

Infection of the human subject has long been known, mainly resulting from accidental transmission among those whose duties bring them into close contact with the pig infection (butchers, veterinary surgeons, laboratory attendants). The records are mainly to be found in the German literature; in 1921, Prausnitz collected those of about one hundred known cases of the disease. It was first studied and described as human 'erysipeloid', by Rosenbach (1884). Following upon introduction of the infection through a wound, there appears within one day or a little longer a painful dark red local swelling which later develops into a rather extensive livid lozenge-shaped patch surrounded by a bright red margin; sometimes the swelling is accompanied by lymphangitis and inflammation of the neighbouring glands. Generalization of infection, rather rarely, takes place, with prolonged fever, rheumatic pains, a scarlatina-like rash, and swelling of the joints. An intestinal form of infection following upon ingestion of raw sausage-meat from pigs dead of swine erysipelas has been described. Dumont and Cotoni (1921) described a peculiar case of meningitis in which they recovered the organism from the cerebrospinal fluid. The presence of the bacillus in the fæces of apparently normal men had been reported. There is almost complete unanimity in the literature in regard to the remarkably efficacious action of anti-swine-erysipelas serum in curative treatment. It is possible that not all cases of

human erysipeloid are derived directly from the pig (Panisset, 1922). In this connection, it is interesting to note that Stefansky and Grünfeld (1930) report a curious outbreak of erysipeloid in two hundred persons in Odessa, in the spring of 1929, in which the disease affected the hands, and was traced to handling consignments of a species of fresh-water fish, which itself was in fresh condition and caused no sign of disease when eaten as food.

REFERENCES.

- CHRISTIANSEN, M., 1919, *Maanedsskr. f. Dyrlaeg.*, **31**, 242.
 COHEN, F., 1927, *Arch. wiss. prakt. Tierhkl.*, **56**, 1.
 CORNELL, R. L. & GLOVER, R. E., 1925, *Vet. Rec.*, **5**, 833.
 DEPARTMENTAL COMMITTEE ON SWINE FEVER, 1896, 1897, see *J. Comp. Path.*, 1897, **10**, 162.
 DUMONT, J. A. & COTONI, L., 1921, *Ann. Inst. Pasteur*, **35**, 625.
 HOPKIRK, C. S. M. & GILL, D. A., 1930, *Vet. Rec.*, **10**, 919.
 MARSH, HADLEIGH, 1929, *J. Amer. Vet. Med. Ass.*, **27**, 724.
 PANISSET, L., 1922, *Rev. gen. Méd. vét.*, **31**, 317.
 PFAFF, F., 1921, *Z. InfektKr.*, **22**, 293.
 POELS, J., 1913, *Folia micr.*, **2**, 1.
 PRAUSNITZ, C., 1921, *Zbl. Bakt.*, Abt. I, Orig., **85**, 362.
 SABELLA, A., 1925, *Zbl. Bakt.*, Abt. I, Orig., **94**, 411.
 SPRYSZAK, A. & SZYMANOWSKI, Z., 1929, *C.R. Soc. Biol.*, Paris, **100**, 1151.
 STEFANSKY, W. K. & GRÜNFELD, A. A., 1930, *Zbl. Bakt.*, Abt. I, Orig., **117**, 376.
 WAYSON, N. E., 1927, *Publ. Hlth. Rept.*, Wash., **42**, 1489.

For further information, the reader is advised to consult the following works of reference :

- ELLENBERGER-SCHÜTZ' *Jahresbericht über die Leistungen auf dem Gebiete der Veterinär-Medizin*, 1881-1929, **1-49**, Berlin, Julius Springer.
 V. HUTYRA, F. & MAREK, J., 1922, *Spezielle Pathologie und Therapie der Haustiere*, 6 Aufl. **1**, Jena, Gustav Fischer.
 KLIMMER, M. & WOLFF-EISNER, A., 1911, *Handb. d. Serumtherapie u. Serumdiagnostik in der Veterinär-Medizin*, Leipzig, W. Kleinhardt (see BAIL, O., *Immunität gegen Schweinerotlauf*, pp. 23-30, and KLIMMER, M., *Impfung gegen Schweinerotlauf*).
 NOCARD, E. & LECLAINCHE, E., 1898, *Les maladies microbiennes des animaux*, Paris, Masson et Cie.
 V. PREISZ, H., 1929, *Handb. d. path. Mikroorg.*, hrsg. Kolle, Kraus u. Uhlenhuth, **6**, Tl. I, pp. 449-482, Jena, Gustav Fischer, and Berlin & Vienna, Urban & Schwarzenberg.

Copies of this volume and of other volumes of the "System of Bacteriology" may be purchased directly from H.M. STATIONERY OFFICE at the following addresses :—ADASTRAL HOUSE, KINGSWAY, LONDON, W.C.2 ; 120, GEORGE STREET, EDINBURGH ; YORK STREET, MANCHESTER ; 1, ST. ANDREW'S CRESCENT, CARDIFF ; 15, DONEGALL SQUARE WEST, BELFAST ; or through any Bookseller.

ABROAD.

Copies of Government Publications are obtainable through the following :—

AUSTRALIA.—Messrs. Angus & Robertson, Ltd., 89, Castlereagh Street, Sydney, N.S.W. ; Messrs. Albert & Son, Ltd., 180, Murray Street, Perth, Western Australia ; Messrs. Oldham, Beddome & Meredith (1932) Pty., Ltd., 36, Elizabeth Street, and 96, Collins Street, Hobart, Tasmania.

CANADA.—The Imperial News Co., Ltd., 235, Fort Street, Winnipeg ; 517, Burrard Street, Vancouver ; The William Dawson Subscription Service, Ltd., 70, King Street East, Toronto, Ontario.

DENMARK.—A. Busck, Kjobmagergade, 49, Copenhagen.

FINLAND.—Akademiska Bokhandeln, Helsingfors.

GERMANY.—Messrs. A. Asher & Co., Behrenstrasse 17, Berlin, W.8.

GOLD COAST.—Wesleyan Methodist Book Depot, P.O. Box 100, Cape Coast ; branches at Accra, Kumasi and Sekondi.

HOLLAND.—N. V. Martinus Nijhoff' Boekhandel, Lange Voorhout 9, 's-Gravenhage.

INDIA.—Messrs. Thacker & Co., Ltd., Bombay ; Messrs. Higginbothams, Ltd., Madras and Bangalore.

IRISH FREE STATE.—Messrs. Eason & Son, Ltd., 40-41, Lr. O'Connell Street, Dublin.

JAPAN.—Maruzen Company, Ltd., 6, Nihonbashi Tori-Nichome, Tokyo.

NEW ZEALAND.—Messrs. Whitcombe & Tombs, Ltd., Auckland, Christchurch, Dunedin and Wellington.

NORWAY.—Cammarmeyers Boghandel, Karl Johans Gate 41-43, Oslo.

SOUTH AFRICA.—The Central News Agency, Ltd., P.O. Box 1033, Johannesburg ; P.O. Box 9, Cape Town ; P.O. Box 938, Durban ; P.O. Box 356, Port Elizabeth ; Pretoria, Transvaal.

SWEDEN.—A-B. C.E. Fritzes Kungl. Hofbokhandel, Fredsgatan 2, Stockholm.

U.S.A.—The British Library of Information, 270, Madison Avenue, New York.

Price £1 1s. 0d. Net.

616.01

DATE OF ISSUE

5989

THIS BOOK must be returned within 3/7/14 V-8
days of its issue. A fine of ONE ANNA per day
will be charged if the book is overdue.

EX-1000ct

25 Feb 76
A402

--	--	--	--	--	--

5989

Class No. 616.01

Book No. 85

Vol 8

Author.....

Title..System of Bacteri
Relation
Medicine

Acc No. 59201

S. Salomon

EXT 300

25Feb76